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MAR 31 1945

CANCER RESEARCH

VOLUME 5
NUMBER 4
APRIL, 1945

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

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THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

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Published by The International Cancer Research Foundation.
Publication Office, 1500 Greenmount Ave., Baltimore 2, Maryland.

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Robert W. Briggs, Business Manager, 1500 Greenmount Ave., Baltimore 2, Md., or 1916 Lincoln-Liberty Building, Philadelphia 7, Pa.

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Entered as second class matter February 12, 1941, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 5 APRIL, 1945 NUMBER 4

The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain

I. Observations on the Adrenal Cortex*

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(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

(Received for publication October 19, 1944)

For some time now studies have been in progress in this laboratory on the relation of gonadectomy to changes in the adrenal cortex and to the development of the secondary sex organs that occurs following certain changes in the cortex.

In the dilute brown strain of mice (JAX dba), nodular hyperplasia of the adrenal cortex following gonadectomy has been observed and described (12, 31, 33). This change occurred in castrated male and female mice both, and preceded and then paralleled an increased development of the secondary sex organs. This development was in a female direction in both sexes, and mammary carcinomas were observed in both males and females that had been gonadectomized at birth. No gonadal regeneration was observed.

It was then shown that these adrenal and sexual changes were not uniform from strain to strain (30). Strains normally with a high, and others normally with a low incidence of spontaneous mammary carcinoma, were observed in this regard. Two strains, dba and C3H, with a high incidence of mammary carcinoma, have more extensive adrenal cortical hyperplasia and accessory sex organ development than does the C57 black strain, which has a low incidence.

The study of adrenal changes and their relationship to accessory sex organs was then extended to include a number of other inbred strains. It was a fortunate circumstance that a number of these had already been developed or were being developed at this laboratory. This report is based upon the extreme dilution strain (JAX ce), in which the adrenal and subsequent sexual responses are characteristically different from those of animals of the dilute brown strain, or of any other inbred strain studied and reported on thus far. It is increasingly evident that the response to gonadectomy varies greatly from strain to strain.

Removal of the testes or ovaries from individuals of the extreme dilution strain of mice leads to carcinoma of the adrenal cortex (32). As will be shown later, these carcinomas metastasize, are transplantable, and then cause increased development of the secondary sex organs of the host. Spontaneous carcinomas of the adrenal cortex are rare in mice, and except for a few cases of carcinomatous changes in our early gonadectomized dilute brown mice accompanying nodular hyperplasia of the adrenal cortex, they have not been observed in the Jackson Laboratory's colonies.

REVIEW OF THE LITERATURE

Slye (19) reported finding 4 adrenal tumors in 33,000 autopsies on mice.[1] These were presumably spontaneous in uncastrated mice, as no mention is made of castration or any other treatment. One was in a male, and 3 were in female mice. Of these 3, the first was a "Cortical adenoma of misplaced interrenal adrenal rest." It was "composed of a solid mass of cells resembling those of the adrenal cortex except in lack of orderly arrangement, closely packed together, and flattening out a thin shell of adrenal cortex." No medullary elements were found. The second tumor was a "Mesothelioma of adrenal with peritoneal metastasis." Microscopically this showed "the usual features of the typical mesotheliomas . . . . with slight

* This work has been aided by grants from the Commonwealth Fund, The Anna Fuller Fund, The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund, and The National Advisory Cancer Council.

[1] The relative percentage incidence of these in males and females is not given.

tendency to alveolar arrangement." The right adrenal was surrounded but not invaded by the tumor, and seemed normal. The retroperitoneal nodes were replaced entirely by tumor tissue. The third growth was a "bilateral malignant mesothelioma." Each adrenal was about the size of the kidney. ". . . . the gross appearance suggested general lymphosarcomatosis . . . . yet the microscopic structure was of an entirely different character." The neoplastic tissue was composed of a solid growth of cells with considerable cytoplasm and nuclei that varied greatly in size. Mitotic figures were abundant. The invaded lymph nodes were largely replaced by tumor cells, and tumor cells were found in the lungs also. The ages of the mice and the strain in which these growths arose were not given. Slye found no records of adrenal cortical neoplasms in mice in the literature.

Gardner (13) reported "adrenal tumors" in 13 mice ovariectomized when 43 to 65 days old, and 495 to 726 days of age at death. These mice were from the third and fourth inbred generations of the NH strain, a strain originating from the combination of 3 inbred strains and "developed for the purpose of producing the highest possible degree of biological variability without the interfering incidence of spontaneous tumor" (20). Some similarity of ancestry, although remote, may exist between the NH and ce strains. Evidence is being gathered on this point. These adrenal tumors were associated with evidences of estrogenic stimulation, as determined by the uterus, pelvis, and mammary glands. The size of the growth could be roughly correlated with evidences of high or low levels of estrogenic stimulation.

Dalton, Edwards, and Andervont (4) have reported a spontaneous, transplantable, adrenal cortical tumor in a female strain C mouse 24½ months of age. They presented evidence that this neoplasm originated from relatively undifferentiated cells of the adrenal cortex. The failure to find metastases from either the original growth or the transplanted tumors suggested that the neoplasm was relatively benign in character. However, the rapid growth rate attained in later generations plus invasion of the capsule indicated a certain degree of malignancy. Careful examination for gross and histologic changes, particularly in the urogenital system and adrenal glands, were made. In female hosts focal edema was noted in the deeper layers of the endometrial stroma; no changes were noted in the genital system of male hosts.

MATERIAL

This report is based upon females of the "extreme dilution" strain of mice, which originated in this laboratory and has now been inbred for from 25 to 30 generations. A preliminary report of the origin, breeding behavior, and tumor incidence in this strain was given at the Third International Cancer Congress, Atlantic City, 1939 (29). Since the proceedings of this congress were not published, some of the findings will be reviewed here.

As now constituted the strain is black agouti, homozygous for the albino series allele c^e, a gene which, when homozygous, causes yellow pigment to be completely suppressed and black diluted to a light tan. The eyes remain dark. The coat of the young is much lighter than that of the adult. The identifying c^e gene in this stock traces through very few hands to a mutant wild male trapped by Mr. J. E. Knight of Weldon, Illinois, in 1920. Detlefsen (7, 8) reported on the genetic behavior of this gene, and our attention was first drawn to some inbred animals carrying it because of evidence indicating that they might develop into a low mammary tumor strain. This has proved to be true. No mammary tumors have been found in several hundred breeding females maintained to advanced ages. In addition we secured evidence that there was a relatively high incidence of gonadal tumors in this strain, ovarian tumors constituting 33 per cent of the total tumors of the female.[2] These mice also failed to reproduce efficiently; over 50 per cent of the females failed to breed when mated brother-sister.[3]

Eaton (9) measured fertility, viability, and growth in 9 inbred strains of mice. One of these (c^d) involved mice selected from strain ce after 15 generations of inbreeding. He found this ce substrain low in its percentage of successful matings, but highest of the 9 in number of young born per litter. The number of young raised and the weight of these at 120 days compared favorably with the other strains tested.

A histochemical difference between "high" and "low" mammary tumor strains was found by Vicari (24). In this study of the amount and distribution of visible cortical lipids in the adrenal glands in relation to mammary tumor incidence she found the ce strain at one month intermediate between the dba strain and C57 blacks both in actual width of the sudanophile zone and width of the sudanophile zone in relation to the whole cortex. Following the work of Miller and Riddle (18) she presented the theory that the visible lipids are storage lipids. Thus ce mice would have less demand for adrenal cortical lipids than those of strain dba, and more than the C57 blacks, if the implications of her interpretation are applied. A peculiar finding of Vicari was that although in strains C57 black, dba, A, N, and C3H the

[2] Data to be reported in detail in a later article.

[3] This failure to reproduce may well be due either to the male or the female or to both. With some brother-sister mating groups only a few females produced young, while in other of these groups either all or no females had offspring.

male had actually as well as relatively more lipid material than the female in the adrenal cortex at 1 month, males and females of the ce strain were the same on a percentage basis, and actually the females had slightly more lipid than the males. The X zone was lipid-free except for a few scattered groups of 2 or 3 large lipid droplets in the females.

This report concerns only animals up to 13 months, that is, 26 virgin females and 34 ovariectomized mice. The number of animals at each monthly interval is given in Tables I and II. The findings for a group of castrated and control males are presented in a companion report (34).

TABLE I: OBSERVATIONS ON STRAIN CE VIRGIN FEMALE MICE

Age, months	No. of mice examined	No. with adrenal tumors	Foci "type A" cells	Foci "type B" cells	X zone
1	2	0	0	0	present
2	3	0	+	0	hyperemic
3	5	0	+	0	"
4	3	0	+	0	narrow
5	2	0	+	0	"
6	1	0	+	0	"
7	2	0	+	0	absent
9	4	0	+	0	"
10	1	0	+	0	"
11	1	0	+	0	"
12	2	0	+	0	"

METHODS

The technic used in this experiment was simple: A group of virgin controls was compared with a group of females ovariectomized when from 1 to 3 days of age.

The mice were anesthetized by chilling in the freezing compartment of an electric refrigerator for from 7 to 10 minutes; placing the mice on dry paper toweling aided in even chilling. For the operation they were placed on a cold glass plate. Small dorsal incisions were made over the region of each ovary with scissors and the ovary and capsule removed with the aid of small forceps; a single silk thread suture was sufficient to close the wounds. The operations were done with the aid of a dissecting microscope (7X ocular and 10X objective). Few mice were lost either as a result of the operation or of removal from their mother for the necessary period.

At 3 to 4 weeks of age the young mice were weaned, earmarked for identification, and maintained in groups of 4 on a diet of Purina fox chow and water.

TABLE II: OBSERVATIONS ON OVARIECTOMIZED STRAIN CE MICE

Age, months	No. of mice examined	Foci "type A" cells	Foci "type B" cells	X zone	No. of adrenals with carcinoma	Adrenal tumor, size *
1	3	absent	absent	present	0	——
2	2	present	"	irregular	0	——
3	3	"	"	"	0	——
4	4	"	present	"	0	——
5	1	"	"	"	0	——
	Mouse no.					
6	P2180	present	present	absent	1	small
6	P2086	"	"	"	1	"
6	P2087	"	"	"	1	"
6	P2088	"	"	"	1	"
6	P2175	"	"	"	1	"
7	P2250	"	"	"	1	"
7	P2252	"	"	"	2	"
7	P2259	"	"	"	1	"
7	P2260	"	"	"	1	"
8	P2257	Observations not possible because of size of tumor			2	medium
9	P2686				2	small
9	P2689				2	"
10	P2182				2	medium
10	P2183				2	"
11	P2273				1	"
12	P2271				2	large
12	P2214				1	medium
12	P2267				2	large
12	P2268				2	"
12	P2659				2	"
12	P2745				2	medium

* Microscopic to 0.5 cm. in diameter = small.
0.5 cm. to 1.5 cm. in diameter = medium.
Greater than 1.5 cm. in diameter = large.

Experimental and control mice up to 13 months of age were selected for autopsy at 30 day intervals and killed by cervical dislocation. Beyond 1 year of age the experimental mice were killed, either because of their large tumors or because they had attained a certain desired age. On account of this method of selection, as well as for ease of analysis, the data are divided according to age.

The adrenals were fixed in a mixture of alcohol, formalin, and acetic acid (11, page 89), embedded in paraffin, sectioned at 8μ, and stained with hematoxylin and eosin.

Gross and microscopic observations were made on both adrenal glands of each mouse. When these were not enlarged sufficiently to give definite indication of extended neoplastic growth, they were serially sectioned.

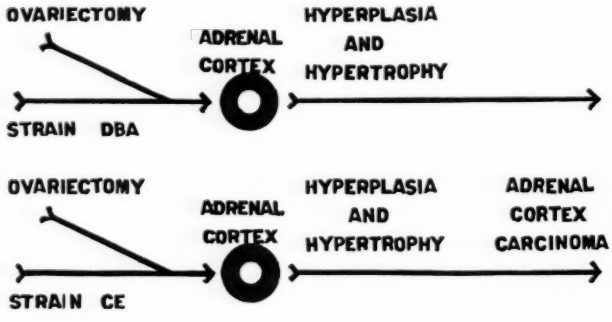


FIG. 1.—Carcinoma of adrenal cortex dependent on (a) gonadectomy of (b) specific strain.

Attempts to transplant a number of adrenal carcinomas were made, and in many cases were successful. This is to be the subject of a later report.

At autopsy a careful search was made to ascertain whether regeneration of the ovaries had taken place, and suspicious areas were sectioned.

Observations on the Adrenal Gland

General.—The normal adrenal gland of the mouse has been often described (11, 16, 25). In the young mouse at 30 days the cortex is divided into the following regions, from capsule to the medulla: zona glomerulosa; zona fasciculata, with a slight irregularity at the base of the columns; and the X zone, a large eosinophilic zone, relatively larger in the female than in the male, interlocking with the medulla. During the next few months the situation varies for males and females because of a difference in degeneration of the X zone, discussed in a later paragraph. In both males and females the X zone eventually disappears entirely, leaving only a connective tissue band between the cortex and the medulla, while the irregularity at the base of the columns of the fasciculata becomes known as the zona reticularis (25, 26). The adrenal cortex without the X zone has been termed the permanent cortex and the X zone the transitory cortex (27). When considering the question of growth, both normal and abnormal, it is of interest to recall that Whitehead (28) found mitotic figures commonest in the cortex immediately beneath the capsule of the gland and throughout the X zone during the first few weeks of the life of the mouse. Later in life mitotic figures were infrequent in all parts of the normal cortex.

X Zone

A. Virgin females.—At 1 month the X zone was intact; a few mitotic figures were present. At 2 months it was slightly hyperemic, with some evidence of cellular shrinkage near the medullary border. Mitotic figures were not seen in X zone cells then or at more advanced ages. At 3 months there was extensive nuclear and cellular shrinkage of X zone cells as well as extensive hyperemia in this zone. This proceeded irregularly from region to region within individual glands. The X zone was narrow for the next few months, and seemed completely gone in most regions at 7 months and all regions at 9 months.

At no time did the X zone occupy so extensive a part of the adrenal cortex as in some adrenals of virgin females from the strains used by Howard-Miller (16) or by Takewaki (22).

When the age at which the X zone degenerated was compared with the observations of Daughaday (5) it was noted that the adrenal glands of mice from the ce strain more closely paralleled those from the dba strain than those of C57 blacks. According to his observations, the X zone in C57 black mice cannot be identified as a distinctive region of the cortex after 100 days, whereas in dba mice it may persist to 210 days or longer.

B. Ovariectomized females.—The X zone at 1 month was similar to that in a control virgin female of the same age. Degeneration was evident at 2 months, and had progressed further than in controls of the same age. This acceleration of X zone degeneration was evident at later periods also, until at 6 months the process was complete. As degeneration proceeded there was more variation from region to region within individual glands than in the glands of control virgin females.

It has been pointed out (6, 27) that involution of the X zone may occur in two different ways; either it becomes vacuolated, the cells being loaded with fatty substances and degenerating *in situ,* leaving a mass of connective tissue stroma; or the cells merely become shrunken and crowded together and finally disappear. Waring (25) emphasized the fact that fatty degeneration is never found in the male. Involution in ce mice was almost entirely without vacuolation

and fatty degeneration. Variation in the method of X zone degeneration exists between strains of mice, since Daughaday (5) found more vacuolation in the high mammary tumor dba strain than in the low tumor C57 black strain.

Beginning at 5 months in ovariectomized females, and at 6 months in control virgin females, groups of cells with yellow pigment and vacuoles, as well as giant cells containing yellow-brown pigment, vacuoles, and 2 to 14 or more scattered nuclei, were found near the cortico-medullary border. Similar giant cells have been described under the term "brown degeneration" (3). These became more numerous in older age groups, but never formed a continuous band or ring. They first appeared when X zone degeneration was nearing completion. It has been reported that the development of these cells tends to be greatest in mice of advanced age, but the time of earliest occurrence was not determined (1). It has been suggested that these cells have some relation to the etiology of mammary gland carcinoma (2), but this has been questioned (1). The extent of development of brown degeneration varies from strain to strain (1, 2, 17). It has been produced by the prolonged application of estrogens (2).

"Type A" cells.—Attention is called in this paper to groups of subcapsular cells that usually have not been emphasized in discussions on the architecture of the mouse adrenal. Localized groups of these cells occurred in both intact and ovariectomized mice, where the cells could be identified on gross and microscopic examination by the time the animals were 2 months of age. The areas became more numerous and more extensive as age advanced.

In the gross they appeared as round, dark areas that seemed to be scattered at random over the adrenal; later, depending on other conditions within the animal, they occupied little or much of its surface. For convenience the cells composing these areas will be termed "type A" cells (Figs. 2 to 5).

Microscopically, foci of type A cells were first found in the immediate subcapsular region of the cortex. The cells were usually ovoid, although they varied from round to slightly fusiform. The nucleus was generally ovoid and relatively large. The cytoplasm stained very slightly and the cell walls were indistinct. These cells seemed to be similar in character but not in extent to the first subcapsular [4] cells described by Zwemer, Wotten, and Norkus (35) for the mammalian adrenal.

Foci of these cells were perhaps the result of an increase in subcapsular cells without subsequent, or at least rapid, differentiation. This interpretation assumes continuity of the cell types from the capsule in the growth of the cortex from without inward.[5]

In older virgin females type A cells were found massed 6 to 8 layers deep under the capsule to the exclusion of other cells. They also surrounded groups of zona glomerulosa cells, and penetrated in groups 3 to 4 cell layers wide between the cell columns of the zona fasciculata. In general, expansion of these areas was inward toward the medulla as age increased (Fig. 4).

In ovariectomized females, foci of type A cells were more extensive at any given age than in virgin females, and also they were definitely localized. In an ovariectomized mouse 4 months of age the cells had massed some 15 layers deep to the exclusion of other types in localized areas. Beginning at 4 months of age foci of type A cells were themselves disturbed by enlarged cells, and at 6 months and later by adrenal cortical carcinomas. At 11 and 12 months only 2 adrenal glands were without these neoplasms; 1 in ♀ P2273 [6] and 1 in ♀ P2214.[6] In both, masses of type A cells were in general limited to the outer third of the adrenal cortex, and were very numerous in this region. In ♀ P2273 [6] the adrenal capsule had been ruptured and type A cells had invaded the surrounding fat.

There was a relation between focal degeneration of the X zone and localized hyperplasia of the subcapsular cells. Type A cells appeared near the capsule over regions where X zone degeneration was rapid. In a few locations, at 2 months, X zone degeneration preceded the appearance of localized masses of type A cells, though at most sites both changes occurred at this age. The relationship was followed until eventually X zone degeneration was general. Both the localized degeneration and the grouping of type A cells were more pronounced in ovariectomized than in virgin females. The cytoplasm of the cortical cells between the type A cells and the degenerating X zone cells stained more deeply with eosin than similar cells in other regions of the same gland. This probably indicated a metabolic disturbance.

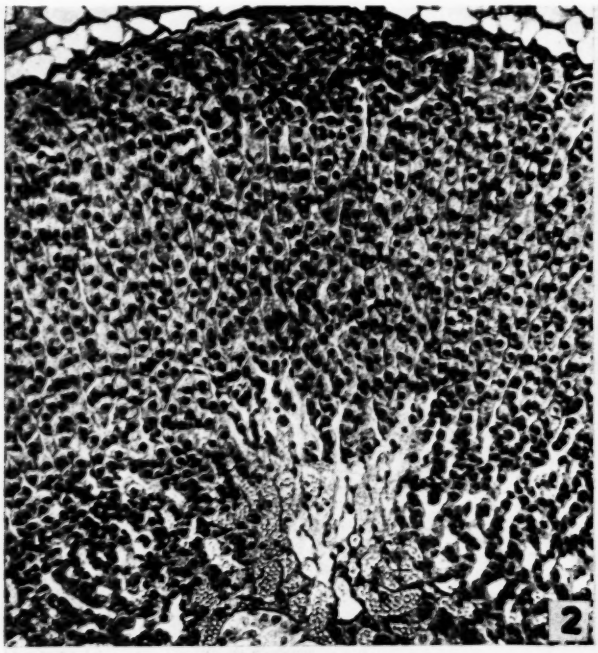
Attention has been called to type A cells not only because (a) they were more prominent in ovariectomized than in virgin mice and (b) more definitely localized in the former than in the latter, but also because (c) they were related to focal changes in the adrenal cortex, possibly to the location of the small cortical carcinomas to be described later.

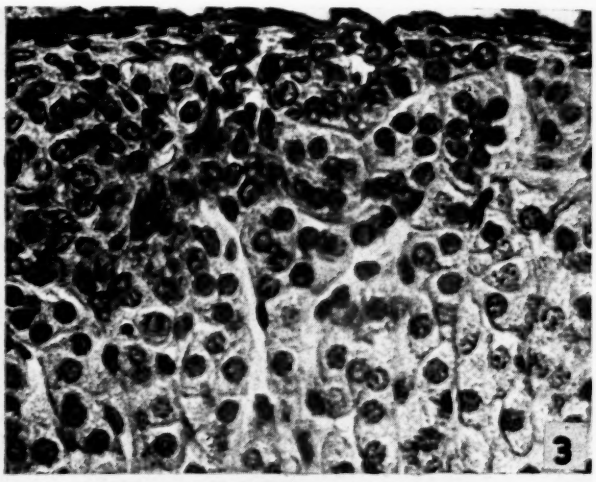
"Type B" cells.—In the adrenal glands of ovariectomized mice, beginning at 4 months of age, certain

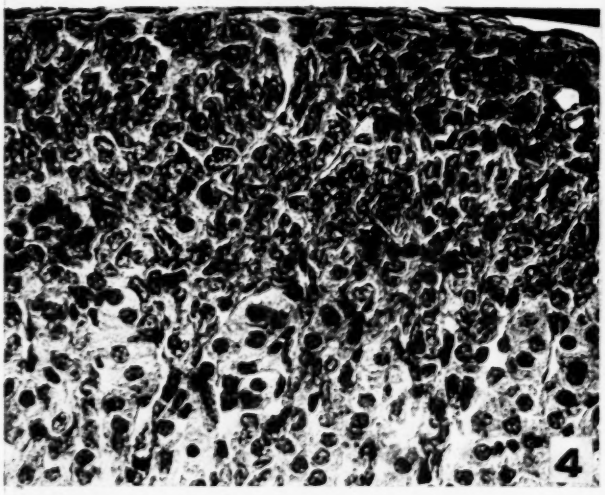
[4] Distinguished from subcapsular cells of the zona glomerulosa described in the same article.

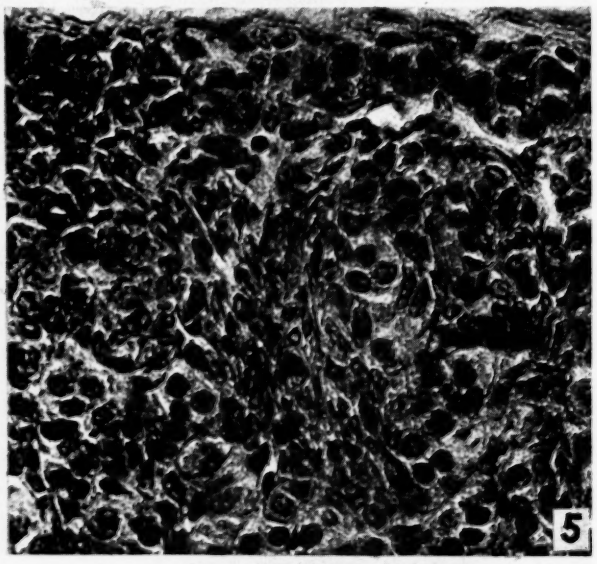
[5] Further study of the method of cell replacement in the cortex of the mouse adrenal seems to be needed to establish the relative importance of different possible methods. Perhaps extensive differentiation of subcapsular cells into other types is not to be expected here.

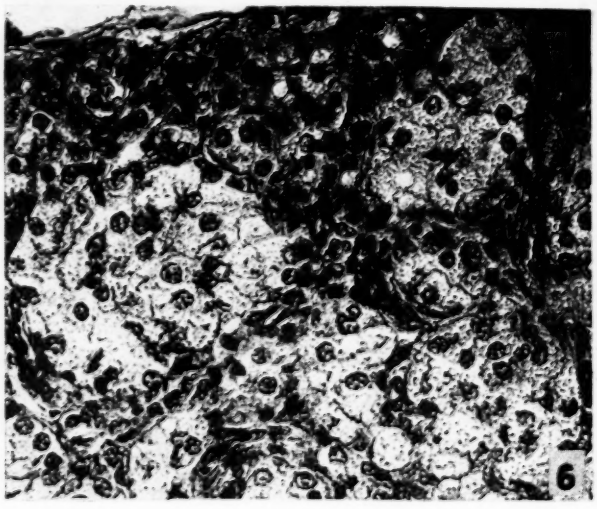
[6] Ovariectomized females.

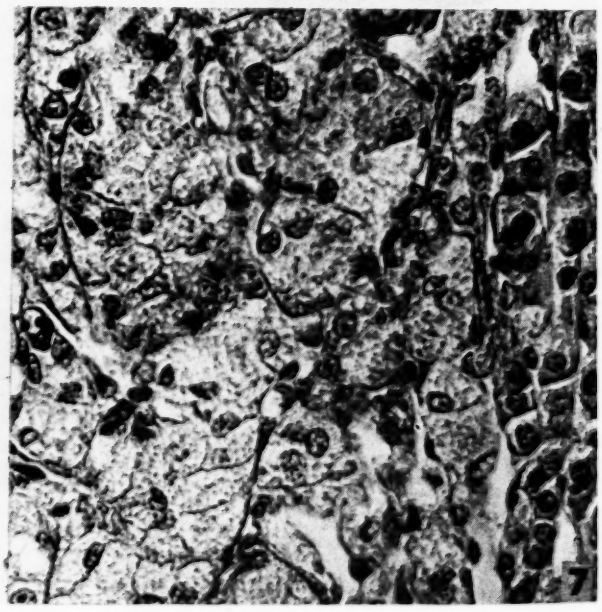












FIGS. 2-7

cells were found greatly enlarged. Their cytoplasm took relatively little stain. Groups of these cells were localized within or close to the groups of type A cells, already described (Fig. 6).

Areas containing these cells could be observed upon gross examination as dense white spots on the surface of the adrenal, encircled at first by the darker looking type A cells. They increased in size as age increased. By 12 months the white color changed to yellowish-brown.

Microscopically, these enlarged cells (for convenience termed "type B" cells) were found localized within or adjacent to masses of type A cells. They were polygonal in shape, irregular in size and arrangement, their cytoplasm stained lightly with eosin, and vacuoles were present. The nuclei were slightly larger than those of the zona fasciculata. When type B cells were in the region normally occupied by zona fasciculata, they did not show the variation in density of staining often observed in the cytoplasm of its own cells.[7] By 6 months all layers of the cortex were interrupted by groups of type A and type B cells, and in a few cases at 6 and 7 months the capsule had been raised by masses of these cells; *i.e.,* the adrenals were nodular.

Areas of type A and type B cells were also noted in our series of gonadectomized dba mice. Extensive masses of these cells formed areas that were described as nodular hyperplasia. In the ce strain these areas, although less extensive than in dba mice, took on new significance because in many cases they were spatially related to and completely enclosed small carcinomatous areas. Fig. 8 shows a small carcinoma among enlarged cells.

Why should the ovariectomized mice have hypertrophy of the adrenal cortex? Applying the speculative method of Tepperman and his associates (23) to an interpretation of the present type of hypertrophy one might assume that (a) there had been, for some reason, an increased inactivation (utilization) of adrenal cortical hormone because of ovariectomy; (b) the fall in adrenal cortical hormone level removed an inhibitory influence on the adrenotropic activity of the anterior pituitary; (c) increased adrenotropic activity led to hypertrophy of the adrenal cortex. The intimate relationship between the pituitary and the adrenal cortex has been reviewed (21).

The antecedents of type B cells are not known; perhaps these originate from glomerulosa cells. At least they were often found in the glomerulosa zone. Another possibility is that they originate from type A cells that preceded them in time of appearance and later were in close spatial relation to them.

[7] It is interesting to note that although Hoerr (15) described a crescentic vacuole toward the medulla in cells of the spongy zone in the guinea pig, it was characteristic of our material from the mouse that the side of the cell toward the capsule stained less intensely than that toward the medulla.

Carcinomas of the Adrenal Cortex

A. Frequency.—Tumors of the adrenal cortex first appeared in 100 per cent of the ovariectomized females at 6 months of age, and were present in 100 per cent of the females in succeeding age groups. In this period, 6 to 12 months inclusive, 21 ovariectomized females were examined (Table II). In the same age group 11 intact females were examined and no adrenal cortical tumors were found. Thus these tumors, a universal characteristic among the older ovariectomized mice, were limited entirely to this group. Among the 21 females with carcinomas the growths were bilateral in 11. Since a number of the growths were found only upon microscopic examination, and no record was kept of right and left adrenals where no tumor was obvious, the frequency of tumors for each side alone was not determined. The frequency of these growths as a whole shows an increase over that previously reported (32) because more small tumors are included.

B. General appearance.—In general the smaller carcinomas were dense, cellular nodules composed of atypical cells showing frequent mitotic figures. They were embedded in the outer region of the cortex, which already was disorganized with groups of type A and type B cells (Fig. 8).

Expansion into the medulla was evident in all cases as the nodules enlarged (Fig. 9). Eventually the medulla and the remaining noncarcinomatous cortical tissue was spread out more or less as a sheet a few

DESCRIPTION OF FIGURES 2 TO 7

Fig. 2.—Increase of subcapsular type A cells in ovariectomized female 2 months of age. Note localized X zone degeneration. Mag. × 135, hematoxylin and eosin. Green filter.

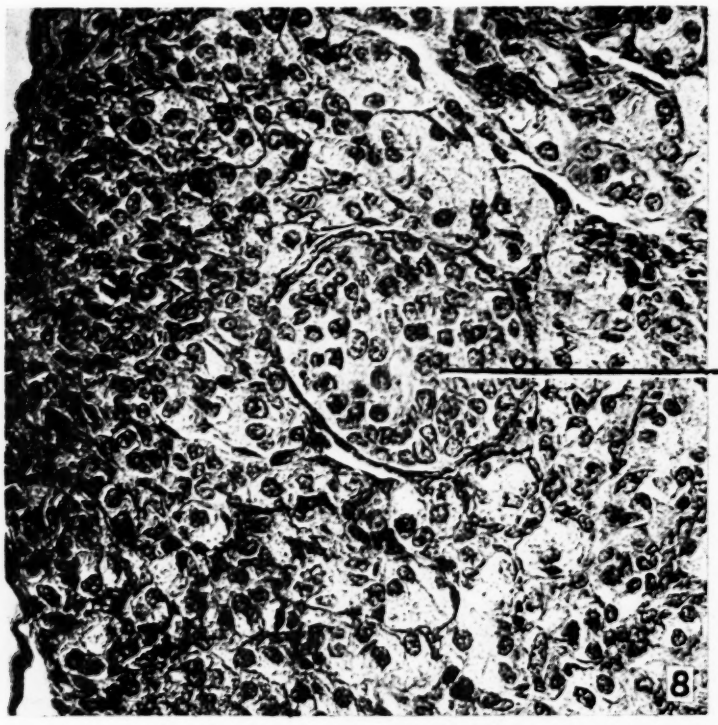
Fig. 3.—Same as Fig. 2, showing type A cells, at left, in more detail. Mag. × 475.

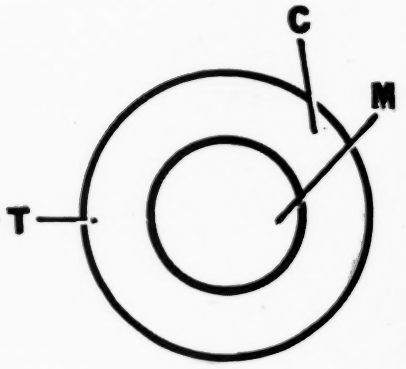
Fig. 4.—Localized type A cells in adrenal cortex of ovariectomized female, P2175, 6 months of age. Mag. × 350.

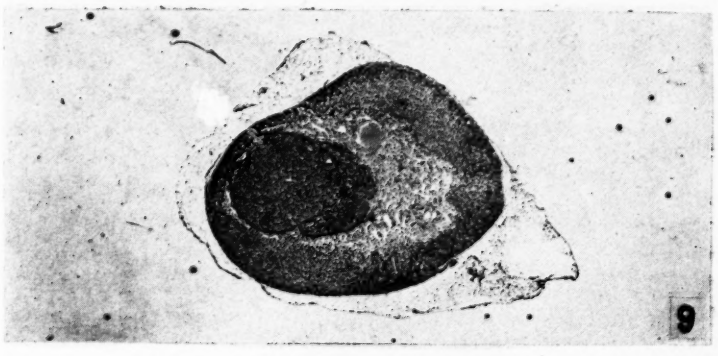
Fig. 5.—Same as Fig. 4, showing type A cells of 6 months old ovariectomized female in more detail. Mag. × 475.

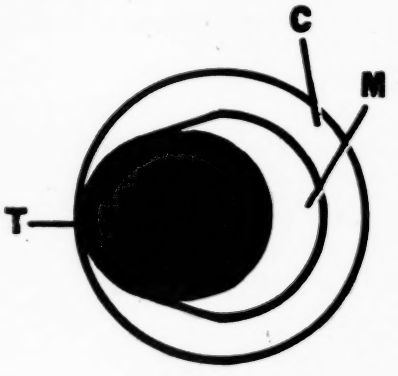
Fig. 6.—Nests of enlarged type B cells in outer part of adrenal, P2260; ovariectomized female 7 months of age. Mag. × 350.

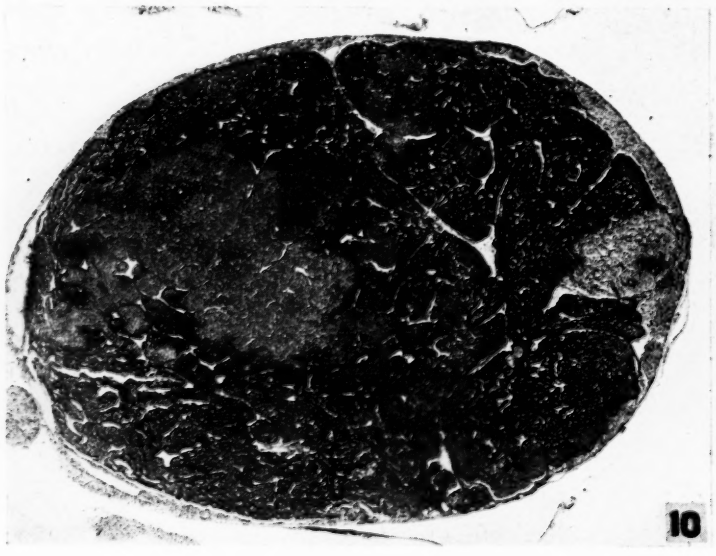
Fig. 7.—Same as Fig. 6. Type B cells with normal fasciculata cells at right. Mag. × 475.

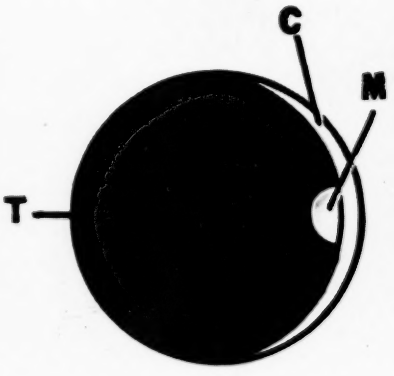












FIGS. 8-10

cells thick; first over much of the tumor and later, because of its large size, over but part of its outer surface (Fig. 10). The growth was fairly well rounded in its earlier stages, but as it attained a size of 1.5 cm. or more in diameter protuberances often developed. The rounded contour was also modified by the pressure of the adjacent kidney. Not only did the kidney modify the shape of the tumor, but the tumor modified the kidney. In some instances this organ was flattened on its anterior surface; in others, partially covered over by tumor, but no invasion of the kidney was noted despite this close spatial relationship. The approximate sizes of the neoplasms, taking right and left tumors together, in the different mice at their autopsy age are given in Table II. It seems evident that a mouse may carry an adrenal growth for many months without its causing death, for none died of cortical carcinoma up to 13 months of age.[8]

A detailed account of the structure of these growths will appear in a future publication.

C. Metastases.—One ovariectomized female at 12 months of age, P2271, had a metastasis to the lung, but none were found elsewhere in this animal, nor were any discovered in other mice at ages from 6 to 13 months. Dalton, Edwards, and Andervont (4) found no metastases from adrenal tumor C-199, and Gardner (13) none in his series of adrenal tumors in NH mice. Ewing (10), however, refers to "bulky metastases" from diffuse adrenal carcinoma in man; Glynn (14) reports for man: "They do not all produce metastases."

DISCUSSION

The removal of ovaries from ce strain female mice was definitely associated with the formation of adrenal cortical carcinoma. *Unless this operation was performed these tumors were absent.* When the ovaries were removed, 100 per cent of the females from 6 to 12 months of age inclusive (21 in number) developed these neoplasms. It seems to have been the history of cancer research that progress has been made, with mice at least, where cancer can be obtained with high frequency. One might include mammary carcinoma, leukemia, lung cancer, and skin cancer, for example, all of which can be readily elicited in certain animals with appropriate technics or experimental influences. It may be expected that our knowledge of cancer will be advanced by the further study of adrenal cortical carcinoma, now that this type of tumor can be produced experimentally with high frequency.

These neoplasms are preceded by the appearance of unusual, localized groups of cells, especially in other parts of the adrenal cortex, the result of increase in both cell size and number. In some cases this process was so extensive at 6 months of age that all cell layers of the cortex were disturbed and the normal contour of the capsule was distorted. Further study of these nodules and their possible relation to the subsequent development of carcinoma, as well as to the failure of carcinoma to appear, as illustrated in ce and in dba mice respectively (Fig. 1), offers interesting possibilities. The importance of the discovery and study of any progressive steps that may lead to cancer is obvious.

When the results of ovariectomy in the ce strain are compared with the results obtained following ovariectomy in other strains of mice, dba and C57 black, for example, a great variation in response is found (30). The problem of the extent and nature of this variation needs more attention. Are the reasons for the differences simple or complex? Are the variations chromosomal or extrachromosomal? If they are extrachromosomal, what is their nature? An attempt to answer some of these questions is being made at this laboratory by the use, as a start, of reciprocal crosses between inbred strains such as dba, ce, and C57 black.

SUMMARY

Two groups of mice belonging to the ce strain, and from 1 to 13 months of age, were autopsied at monthly intervals. The groups consisted of : (a) intact virgin females, and (b) gonadectomized females. Ovariectomy was performed when the mice were from 1 to 3 days of age. Adrenal cortical carcinomas were found only in the ovariectomized mice. These occurred in 100 per cent of the 21 females, 6 to 12 months of age inclusive, and in none of the 26 intact mice of this strain. Progressive changes in the adrenal cor-

[8] Translating this roughly into terms of the human adult this would mean that should a patient have a tumor of this type and rate of growth he might be expected to survive at least 15 or 20 years. It is known, however, that children with untreated adrenal tumors often live only a very few years, and Ewing (10) states that most adrenal carcinomas progress rapidly from the first symptom.

DESCRIPTION OF FIGURES 8 TO 10

FIG. 8.—Small carcinomatous nodule in adrenal cortex of ovariectomized female, P2250, 7 months of age. Note type A cells toward periphery of cortex, and type B cells near tumor. Mag. × 350.

FIG. 9.—Carcinoma in adrenal of P2087, ovariectomized female 6 months of age. Origin of tumor interpreted as being almost at right angles beneath section shown. Mag. × 18.7.

FIG. 10.—Carcinoma in ovariectomized female 12 months of age. Note medulla at right near the remaining cortex. Two types of cells within carcinoma. Mag. × 18.7.

tex preceding the appearance of the carcinomas are described.

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The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain

II. Observations on the Accessory Sex Organs*

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(Received for publication October 19, 1944)

Some effects of ovariectomy on the growth of the adrenal glands have been described in the preceding part of this report (21). The purpose of the present paper is to present observations on the accessory sex organs following ovariectomy. To aid in doing this, comparisons are made between virgin females and ovariectomized females.

Evidence that the accessory sex organs of certain female mice do not remain inactive following ovariectomy has recently appeared.

Perry (14) found positive vaginal smears in 2 untreated spayed mice that had been kept in a box with spayed females receiving injections of 1, 2, 5, 6-dibenzanthracene, which was considered slightly estrogenic. Smears were taken twice weekly. The 2 mice were beyond 9 months of age when positive smears were found.[1] The strain of mice used was not mentioned.

Loeb and Kirtz (10) reported observations on 5 mice of the D strain that had been ovariectomized at about 5 weeks of age and had received 3 to 5 transplants of anterior pituitary 3 weeks later. When these mice were examined at 11 to 12 months of age, a mammary tumor was found unexpectedly in 1 of them. The uterus, vagina, and mammary glands were all well developed, suggesting the action of ovarian hormones. It was thought that some regenerative or compensatory process had taken place, which caused a response of the accessory sex organs to the transplants of anterior lobe similar to that in normal ungonadectomized bearers of transplants. No ovarian tissue was found.

Woolley, Fekete, and Little (20) found that JAX dba mice ovariectomized within a day or two after birth, had uniformly well developed accessory sex organs late in life. This was interpreted to mean that the adrenal cortex, which was enlarged by nodular hyperplasia, simulated the action of the ovaries.

Similar observations by Gardner (7) have already been referred to (21).

MATERIAL AND METHODS

The data include 26 virgin females and 34 ovariectomized females of the "extreme dilution" strain (JAX ce) of mice, already described in the preceding paper (21).

Gross as well as microscopic observations were made on many of the organs, including the submaxillary, mammary, and pituitary glands, and the uterus and vagina. Sections of all were examined microscopically with the exception of the mammary glands. These were studied in whole mount; a few critical ones microscopically. The methods of fixation, sectioning, and staining have been described (21).

Observations on the Sex Organs

Uterus.—The uteri were studied in cross section, care being exercised to obtain the section as nearly as possible midway between the cervix and the region of the fallopian tube. In the virgin females (Table I) the uteri were small in diameter and undeveloped at 1 month; of medium size at 2 and 3 months, and variable in size but considered normal in subsequent periods. The uterine glands were small at 1 and 2 months and of normal size in later months; one exception was ♀ P2351 (Table II) at 10 months, in which the uterine glands were large and cystic. P2350 at 11 months had very large but not cystic glands. The stroma of the endometrium was dense and cellular at 1 and 2 months and less cellular, although variable according to the stage of the cycle, at later

* This work has been aided by grants from the Commonwealth Fund, The Anna Fuller Fund, The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund, and The National Advisory Cancer Council.

[1] At this age the positive smears might have been due to stimulation from nodular hyperplasia of the adrenal cortex (6, 20), an interpretation that was not suggested.

TABLE I: OBSERVATIONS ON VIRGIN FEMALE MICE OF THE ce STRAIN

Age, months	Number examined	Number with adrenal tumors	Uterine diameter	Vaginal epithelium	Submaxillary gland	Mammary glands: Duct length	Mammary glands: End buds	Mammary glands: Alveoli
1	2	0	small	normal	♀-type	short	present	absent
2	3	0	medium	"	"	"	"	"
3	5	0	"	"	"	medium	"	"
4	3	0	large	"	"	"	absent	"
5	2	0	"	"	"	"	"	"
6	1	0	"	"	"	"	present	"
7	2	0	"	"	"	"	absent	"
8	0	0	———	———	———	———	———	———
9	4	0	large	normal	♀-type	long	absent	absent
10	1	0	"	"	"	"	"	"
11	1	0	"	"	"	"	"	"
12	2	0	"	"	"	"	"	"

TABLE II: OBSERVATIONS ON OVARIECTOMIZED MICE OF THE ce STRAIN

OBSERVATIONS ON MICE WITHOUT ADRENAL CORTICAL CARCINOMAS

Age, months	Number examined	Uterine diameter	Vaginal epithelium	Submaxillary gland	Mammary glands: Duct length	Mammary glands: End buds	Mammary glands: Alveoli
1	3	small	2	♀-type	short	absent	absent
2	2	"	"	"	"	"	"
3	3	"	"	"	"	"	"
4	4	"	"	"	"	"	"
5	1	"	"	"	"	"	"

OBSERVATIONS ON MICE WITH ADRENAL CORTICAL CARCINOMAS

Age, months	Mouse number	Uterine diameter	Vaginal epithelium	Submaxillary gland	Mammary glands: Duct length	Mammary glands: End buds	Mammary glands: Alveoli	Carcinoma, size *
6	P2080	small	2	(changing to ♂-type)	short	absent	absent	small
6	P2086	"	"	"	"	"	"	"
6	P2087	"	"	"	"	"	"	"
6	P2088	"	"	"	"	"	"	"
6	P2175	"	6 to 8	"	"	"	"	"
7	P2250	"	2	"	"	"	"	"
7	P2252	"	"	"	"	"	"	"
7	P2259	"	———	"	"	"	"	"
7	P2260	"	2	"	"	"	"	"
8	P2257	medium	[cornified 11-12]	"	medium	present	"	medium
9	P2686	small	2	♂-type	short	absent	"	small
9	P2689	"	2-3	"	"	"	"	"
10	P2182	medium	[cornified 11-12]	(changing to ♂-type)	long	present	"	medium
10	P2183	"	[cornified 10-14]	♂-type	"	absent	"	"
11	P2273	"	[cornified 10-12]	"	"	"	"	"
12	P2271	large	[cornified 8-14]	"	"	present	present	large
12	P2214	medium	[cornified 12-14]	"	"	absent	absent	medium
12	P2267	large	6-7	"	medium	"	"	large
12	P2268	"	10-12	"	long	"	"	"
12	P2659	"	7-10	"	medium	"	"	"
12	P2745	"	[cornified 8-10]	"	"	"	"	medium

* Microscopic to 0.5 cm. in diameter = small.
0.5 cm. to 1.5 cm. in diameter = medium.
Greater than 1.5 cm. in diameter = large.

months. ♀ P2570 at 12 months had the most hyaline connective tissue in the series. There was less variation in this respect than in the ovariectomized group. The uterine epithelium was tall columnar in ♀ P2570. Beginning at 3 months it was tall in some mice (in P2120 at 3 months, for example). At 3 months and later the height of the uterine epithelium varied normally according to the stage of the estrous cycle. The myometrium became prominent, with an increase in the size of its fibers as the uterus enlarged, and remained so in all animals with the exception of ♀ P2570 at 12 months. This mouse, because of the greatly expanded uterus, had a very thin and indefinite myometrium. In the younger age groups the uterus had a slit-like lumen; at 11 and 12 months the lumen was regularly more branched than at earlier ages.

In the ovariectomized mice the uterus remained undeveloped, but otherwise very similar to that of the virgin females at 1 month of age up to the eighth month, when ♀ P2257 [2] showed a definite increase in uterine diameter. The cells lining the lumen in this mouse were tall columnar (Fig. 8) and the uterine glands were unusually enlarged. The endometrial stroma was less cellular than in other individuals observed up to this age and the myometrium well developed. This mouse had two carcinomas in the adrenal cortex, which were classified as of medium size on the scale used in Table II, and thus composed the largest mass of tumor recorded up to this age. ♀ P2182 [2] at 10 months had a uterus similar to that just described except for its larger glands. Both P2257 and P2182 had vaginal epithelium that showed cornification, indicating that they had reached a period of estrus. All uteri of mice 10 months and older had diameters definitely larger than that of the normal strain ce mouse during the first few months of life. In general, the uteri were of nearly normal size for virgin females of a similar age. ♀ P2271,[2] an exception, had a much larger uterine diameter than did any of the virgin females. Greater differences existed from individual to individual in the degree of fiber formation in the endometrial stroma than in the virgin females. No test to differentiate reticulin and collagen was made. Variations in the stroma are shown in Figs. 8 and 9, which are from P2257 at 8 months and P2659 at 12 months, respectively. The uterus of P2271, the only individual that had alveolar growth of the mammary gland, had no nuclei in the uterine stroma that might indicate the corpus luteal type of hormone, as reported by Hooker (8). In summary it may be said that the uterine diameter was closely correlated with the size of the adrenal tumor up to the 13th month.

Vagina.—In the intact virgin mice the vaginal epithelium was normal. Growth and development was apparent at 1 month and thus was detected before uterine reaction. Mucus was found in the vaginal epithelium at 2 months. The first mouse to show cornified vaginal epithelium was 3 months old; in others of this age group the epithelium was from 8 to 14 cells thick. Beyond this age normal variations were found according to the stage of the cycle at which the mouse was autopsied.

In the ovariectomized females the vaginal epithelium was typically 2 cell layers deep and undeveloped up to the age of 6 months. P2175, autopsied at 6 months of age, had vaginal epithelium 6 to 8 cell layers deep with nuclear evidence of rapid growth. Here, as in the control group, evidence of increased development could be noted in the vaginal epithelium before it could be detected in the uterus. P2175 had 1 small carcinoma in the adrenal cortex. P2257, autopsied at 8 months, had vaginal epithelium 11 to 12 cells thick, with a cornified layer. This was the first mouse in the ovariectomized series to show cornified vaginal epithelium. P2257, as noted previously, had the largest mass of adrenal tumor recorded up to this age. All the mice at 10 months and beyond showed thickened vaginal epithelium, the condition of which indicated that they were in various stages of estrus. The thickness of the epithelial layer and whether or not cornified cells were present are shown in Table II. A study of the possible cyclic behavior of the vaginal epithelium was made in only 1 individual in this series; smears were studied on P2659 for 26 days, beginning at 9 months of age.[3] The percentage of cornified epithelial cells, nucleated epithelial cells, and leukocytes was calculated. The percentage of cornified cells was low throughout the 26 days except on 2 occasions, when they reached 69 and 48 per cent of the total. The percentage of cornified cells was at all times too low to be characteristic of a normal cycle. Evidence of full cornification of the vaginal epithelium was found, however, in sectioned material; in 1 animal at an age younger than that given above.

Mammary glands.—The mammary glands of 23 virgin control females were studied, almost entirely in whole mounts. To prepare them for observation the skin together with the mammary glands was removed, starting from the dorsal surface; care was exercised that the axillary and inguinal fat pads, which normally contain mammary tissue, should remain with the skin. The flap thus obtained was next pinned out flat on a paraffin surface, fixed, cleared in xylol, and examined with dissecting binoculars. When questions requiring more detailed observation arose the glands were removed from the skin and

[2] Ovariectomized females.

[3] Miss Aurelia Raciti, of Barnard College, a summer student at this laboratory, assisted in the study.

sectioned for microscopic study. The whole series was prepared and saved so that direct comparisons could be made at one time.

animal killed at 1 month, in 1 of 3 killed at 2 months, in 4 of 5 killed at 3 months, and in 1 killed at 6 months. Although other animals were in estrus, as

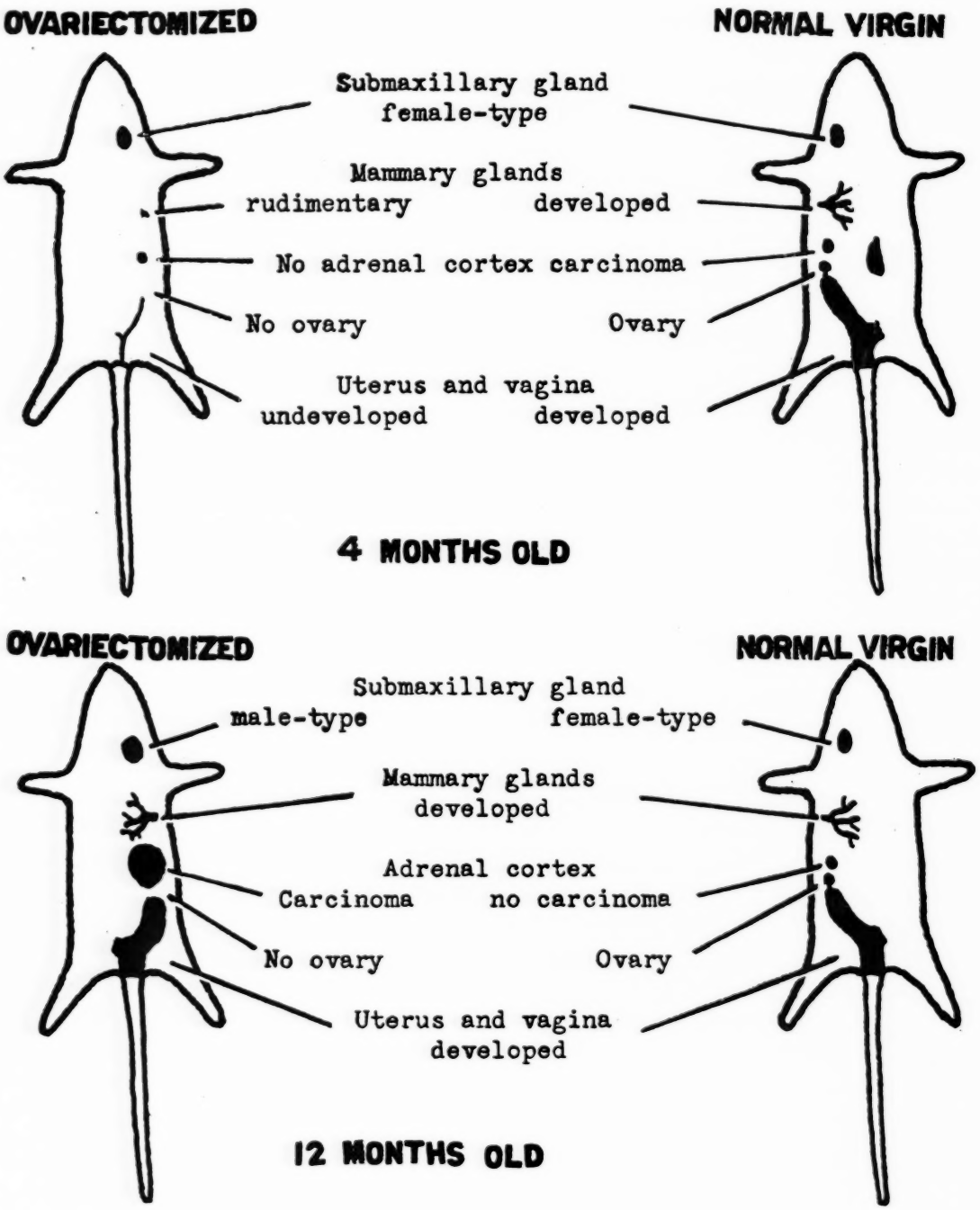


FIG. 1.—Diagrammatic representation of ovariectomized and virgin mice of the cc strain at 4 months and at 12 months of age.

The general findings are listed in Table I. All glands were recovered in each animal. Those of any one individual throughout the series were in general of normal uniformity. The mammary ducts were short for the first 2 months, and of medium to full length up to the ninth month. Beyond this age they were all classed as long. End buds were found in the 1 checked by the vaginal epithelium, they did not exhibit end buds. It has been observed by others that these proliferative changes are more obvious during the first few cycles than later, when the growth of the duct system is more extended (16). Small lateral branches on the mammary glands were not numerous until the ninth month and beyond. No alveoli were

observed in any of the animals. Localized nodules of hyperplastic tissue were not found in any of the glands.

The mammary glands of 32 ovariectomized females were similarly examined (Table II). Without exception the ducts were short unless the animal had a medium or large amount of adrenal cortical tumor, in which case there was extended duct growth. Mammary duct extension is a condition normally associated with estrogenic hormones (1, 2, 15, 17). Fekete, Woolley, and Little (6) found that the mammary glands of ovariectomized dilute brown mice had extended mammary ducts at an age comparable to that at which it occurred in ovariectomized mice of the ce strain without adrenal cortical carcinomas. Nodular hyperplasia of the adrenal cortex was present in these ovariectomized females.

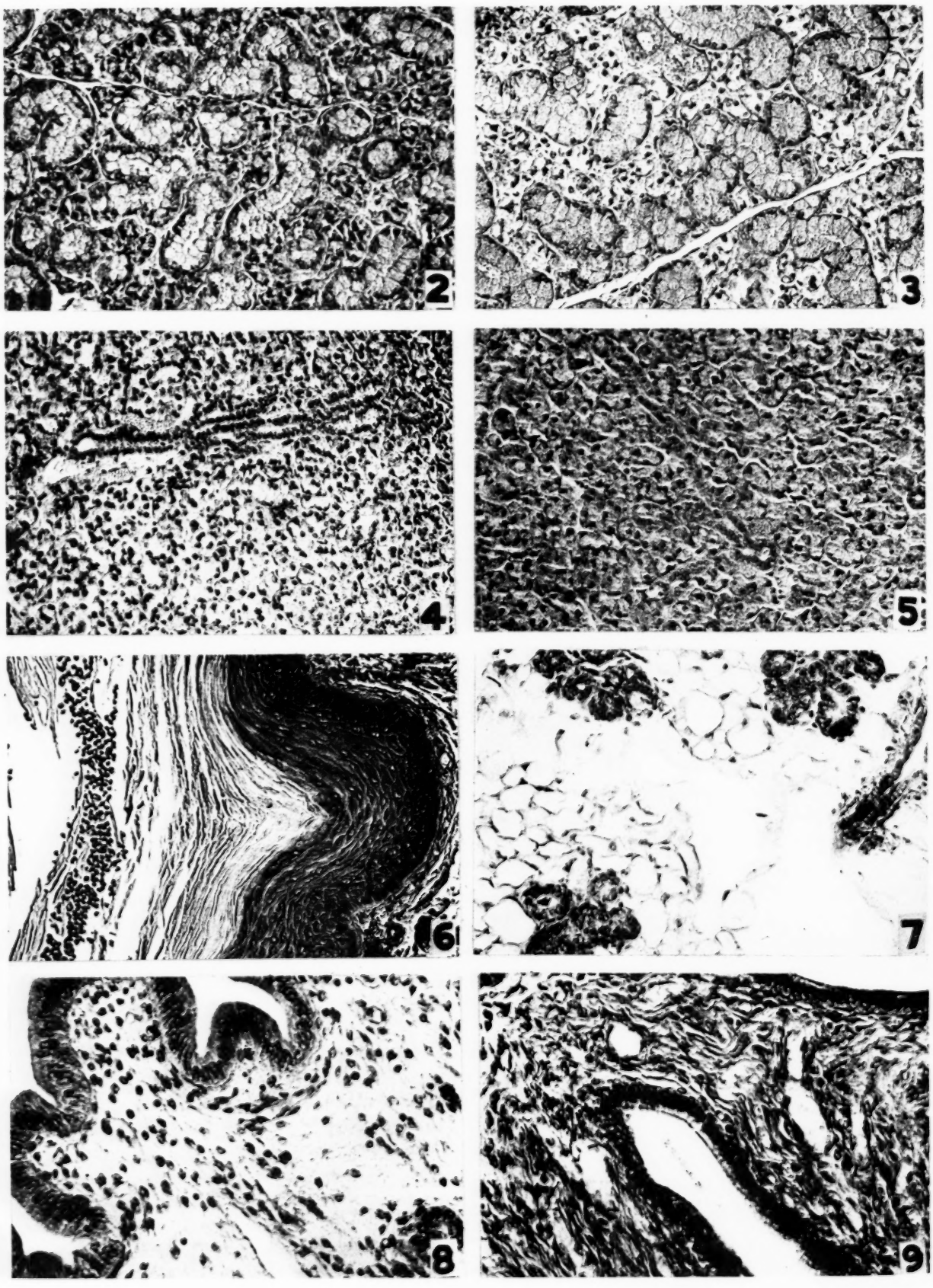
End buds, such as those present in one mouse at 8, in one at 10, and in one at 11 months,[4] have been found associated with proestrus and estrus (2, 4, 16). These proliferative changes are normally more obvious during the first few estrous cycles in intact females than later, when the duct system is more extended (16). In the ce strain they appeared several months later in the ovariectomized than in the control group. P2267, killed at 12 months, had many small branches in the mammary gland similar to those observed in virgin females at the more advanced ages. This mouse had 2 tumors in the adrenal cortex, which together made a large amount of neoplastic tissue.

Alveolar structures were found in only 1 mouse, P2271 (Fig. 7), killed at 12 months. Microscopic examination of a gland showed that the lobules had enlarged somewhat. Fluid was found in the lumen. The appearance of the lobule-alveolar system in mouse P2271, a condition not found in the control series, requires further explanation than is necessary to account for the duct extension alone. Turner and his associates (15) showed that the mammary development normally occurring during the first half of pregnancy can be stimulated experimentally by the simultaneous injection of estrogen and progestin. Burrows (3) arrived at the conclusion that the action of estrogen on the mamma of the female mouse is confined to preparing it so that lactation may be brought about subsequently by other hormones. Work with the male mouse has shown that the ovarian hormones act through the pituitary; *i.e.,* they are not effective in hypophysectomized animals. Mixner, Lewis, and Turner (11) presented evidence of the presence of a lobule-alveolar factor of the pituitary different from the ether-soluble extract that stimulates extensive duct growth. It was suggested that the pituitary merely mediated the action of the ovarian hormones. It has been found that growth of the lobule-alveolar system of the mouse can be stimulated in castrated female mice with pregneninolone, a synthetic progesterone-like compound. Only one-fifth as much was required if simultaneous injection of estrogen was used. Mixner and Turner (13) assayed desoxycorticosterone acetate, dehydroandrosterone, diethylstilbestrol, acetoxypregnenolone, methyltestosterone, testosterone, and testosterone propionate and compared these with progesterone for their ability to initiate mammary lobule-alveolar growth in the ovariectomized female mouse. With progesterone as a standard, pregneninolone had an activity of approximately one-half, desoxycorticosterone acetate and dehydroandrosterone of one-third, diethylstilbestrol of one-fourth, acetoxypregnenolone of one-sixteenth, and methyltestosterone of one-twenty-fifth. Testosterone and testosterone propionate in doses up to 10 mgm. failed to stimulate lobule-alveolar growth. From this brief review it seems evident that it will not be possible by observation alone to arrive at very definite conclusions in regard to the cause of the mammary alveolar development in ovarietomized mouse P2271, though it would seem that testosterone and testosterone propionate can be eliminated from the picture. It is also evident that ovariectomized mice of the ce strain vary in the way that their mammary glands are affected after the occurrence of adrenal cortical carcinoma.

Pituitary.—The findings for the pituitary, similar for both castrated males and ovariectomized females, are discussed in a companion report (22).

Ovaries.—The ovaries of 24 control females were examined. At 1 month the ovary was small, with many follicles throughout. There were no corpora lutea and the stroma was scanty. At 2 months there were a few corpora lutea, many follicles, and scant stroma, although there was more stroma than at 1 month. At 3 months the ovary was approximately three-fifths the diameter of an ovary at 12 months and a much smaller part was occupied by follicles than up to this time. Corpora lutea were present. The center of the ovary had blood-filled sinuses and the stroma had increased greatly. At 4 months and at later periods the area occupied by follicles became progressively less and degenerating follicles more numerous. Masses of large yellow cells began to take a more and more important place in the ovarian stroma. Progressive changes were: (a) a decrease in areas of the ovary occupied by follicles; (b) at first an increase and then a decrease in the area of the ovary occupied by corpora lutea; (c) increase in the number of degenerating and degenerated follicles;

[4] The vaginal epithelium of all 3 of these animals had cornified cells.



FIGS. 2-9

(d) an increase in stroma as well as in nests of large yellow cells within this stroma.[5]

Submaxillary gland.—It has been shown independently by Lacassagne (9) and by Fekete (5) that the submaxillary glands of the mouse show sex dimorphism. The central intralobular ducts of both sexes are lined by rodded epithelium. In the adult female this lines also the terminal tubules into which the central intralobular ducts divide. In the adult male the lining of the terminal tubules and some of the alveoli opening into them consists of tall columnar epithelial cells with the nuclei near and often flattened against the bases.

The submaxillary glands of the control females were all of typical female type. The cells of the terminal tubules were somewhat larger, primarily because of an increase in cytoplasm. At the more advanced ages the nuclei remained in a more or less central position. The tubules never occupied as high a percentage of total gland area as in the male series (Fig. 5). In the gross the female submaxillary glands could be differentiated from those of the males by the fact that they were a rich red rather than whitish in color, and smaller in size.

The submaxillary glands of 29 castrated females were examined (Table II). They were essentially of the female type in their morphology during the first 5 months, but beginning at 6 months were more and more of the type found in young, intact males of this strain. At first the changes were not uniform throughout the gland. In certain tubules, however, the nuclei had taken a basal position and the cells had become tall columnar. The proportion of the total area occupied by the tubules was similar to that in the male at 1 month of age. It is interesting that in a female 6 months old with a tumor nodule only 8 to 10 cell layers in diameter, P2180, as well as in those with larger growths in the adrenal cortex, submaxillary glands had begun to take on more of the male morphology. At 7 months they were definitely more male in type, as was shown mostly by the terminal tubules. P2257 at 8 months had a submaxillary gland of the female type (Fig. 4), well developed sex organs, and a moderately large amount of adrenal tumor tissue. At 9 months the submaxillary glands were similar in type to those of an intact male about 2 months old. Some variation existed from animal to animal in regard to degree of male type structure assumed. This is shown also in the mice at 10 months; for example, where the gland of P2182 was only slightly male in type while that of P2183 was similar to that of a male 1 or 2 months of age. Both had adrenal tumors of medium size, and cornified vaginal epithelium. The latter also had mammary end buds, indicating rapid proliferation. At 11 months the submaxillary glands had tubules that occupied such a large area as to give the appearance of those of an intact male of the ce strain about 3 months of age. This condition held also for mice at 12 months. P2659 at 12 months (Fig. 3) exceeded normal males of 11 and 12 months (Fig. 2) in regard to the increase in tubule size.

[5] This is a very incomplete description of a series of interesting characteristics of the ovary. A more comprehensive and analytical account of these will be published later.

DISCUSSION

The development of accessory sex organs became first evident, and then pronounced, with the appearance and growth of adrenal tumors. Evidence has been presented that estrogenic and androgenic effects at least have occurred, in some instances simultaneously within individual animals. Support has been given recently to the concept that the adrenal cortex produces various types of hormones. Compounds with entirely different pharmacological actions, such as corticosterone, progesterone, and adrenosterone have actually been isolated from the gland (18). If tumor cells follow the pattern of the secretory action of their parent cells, as neoplastic cells to some extent repeat the pattern of their parent structure, one would expect numerous hormone variations in adrenal cortical tumors. Further biological and biochemical study will show the extent to which this occurs.

SUMMARY

Two groups of mice belonging to strain ce, and from 1 to 13 months of age, were autopsied at monthly in-

DESCRIPTION OF FIGURES 2 TO 9

FIG. 2.—Submaxillary gland of normal male mouse, P2317, of the ce strain, 12 months old. Mag. × 150.

FIG. 3.—Submaxillary gland of ovariectomized female, P2659, 12 months old. Male type. Female with large adrenal cortical carcinoma. See also Fig. 9. Mag. × 150.

FIG. 4.—Submaxillary gland of ovariectomized female, P2257, 8 months old. Female type. Female with medium large adrenal cortical carcinoma. Mag. × 150.

FIG. 5.—Submaxillary gland of intact female, P2350, 11 months old. Mag. × 150.

FIG. 6.—Vaginal epithelium showing cornification. Ovariectomized female, P2257, 8 months old. Mag. × 150.

FIG. 7.—Mammary gland from ovariectomized female, P2271, 12 months old, showing alveoli. Mag. × 190.

FIG. 8.—Central region of uterus of ovariectomized female, P2257, 8 months old. Note tall uterine epithelium. Mag. × 150.

FIG. 9.—Central region of uterus of ovariectomized female, P2659, 12 months old. Compare stroma with that in Fig. 8. Uterine gland in center of field; lumen at upper right. Mag. × 150.

tervals. The groups consisted of (a) intact virgin females and (b) females gonadectomized when from 1 to 3 days of age. Growth and development of the accessory sex organs occurred following the appearance of adrenal cortical tumors in the ovariectomized females. Effects attributable to estrogenic and androgenic hormones were recorded.

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The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Male Mice of the Extreme Dilution Strain*

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(Received for publication October 19, 1944)

The effects of castration on the adrenal glands and accessory sex organs in mice of the ce strain are reported in this paper. Studies with ovariectomized mice of this strain are presented in companion reports (23, 24). Cancer of the adrenal cortex appeared following gonadectomy, but was not found in intact mice. Accessory sex organ development was observed in castrated males following the appearance of adrenal cortical tumors.

The report of only one such growth in a male mouse has been found in the literature. Slye (13) reported what seemed to be a "typical mesothelioma of the adrenal in an early stage of malignancy." This male mouse had in place of the right adrenal a spherical mass 5 mm. in diameter, which was completely encapsulated and did not involve the kidney. Microscopically the nodule was found to contain no remains of adrenal tissue, but consisted of a solid tumor made up of masses of large cells with considerable cytoplasm and large oval or spherical nuclei, arranged in atypical alveoli or broad bands with a very small amount of stroma containing thin-walled blood vessels. No mention was made of castration or other treatment of this mouse. The age or strain was not given.

Spiegel (14) noted tumors of the adrenal cortex and a masculinizing effect of these growths in 3 old guinea pigs that had been castrated shortly after birth. Changes in the seminal vesicles, prostate, and penis were given as evidence for a masculinizing effect.

MATERIAL AND METHODS

This study is based on an inbred strain of mice, strain JAX ce, much of the available information regarding which has already been presented (23).

Male mice castrated when 1 to 3 days of age were compared with intact male mice. The 39 castrated and 33 intact animals were killed at monthly intervals beginning at 1 month and ending with the 12th. The number autopsied at each age is shown in Tables I and II.

The method of castration was similar to the method of ovariectomy previously described (23), except that in the case of the males a ventral incision was made just cephalad to the genital papilla and both testes together with their epididymides were removed.

At autopsy the adrenal glands, pituitary, seminal vesicles, prostate, and testes (when present) were saved for microscopic examination. Unless the adrenal glands were greatly enlarged by neoplastic growth they were studied in serial section. Sections of all adrenal glands were examined microscopically. The mammary glands were studied as whole mounts; if some region required more detailed observation, this part was removed and sectioned for microscopic examination.

The method of fixation, sectioning, and staining has been described (23).

OBSERVATIONS ON THE ADRENAL GLANDS

The system of nomenclature already employed for the adrenal cortex (23) will be adhered to throughout this paper.

Normal intact males.—Both right and left adrenals were examined. No cortical carcinomas were observed in this series. The X zone was narrow in the male at 1 month, degenerating during the second and third months, and absent in succeeding months. In a series studied by Waring (17)[1] the X zone of the male reached its maximum development by 21 days, then to about 30 days it remained constant, and by about 40 days it had ceased to exist as an organized layer.

Areas of type A cells were evident in some of the mice at 3 months, and increased in extent during succeeding months, but at no time were they as extensive as in intact females of the same age.

Castrated males.—All the adrenal glands up to 11 months of age were examined in serial sections,

* This work has been aided by grants from The Commonwealth Fund, The Anna Fuller Fund, The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund, and The National Cancer Council.

[1] No strain specified.

as were those beyond this age if they were not greatly enlarged by neoplastic growth. As with ovariectomized females (23), in animals beyond 1 month of age and before neoplastic growth had disturbed the normal adrenal structure, localized areas of type A cells were evident. Beginning with the group autopsied at 6 months, type B cells became prominent, and were extensive in mice autopsied during the next few months (see Table II). In later months neoplastic growth prevented accurate estimation of the extent of these cells. Foci of type A cells had a much less disturbing effect on the X zone in the intact series than in the gonadectomized group.

Early removal of testes in mice of the ce strain → led to → adrenal cortical tumors → led to → accessory sex organ development of ♂-type and (or) ♀-type

FIG. 1.—Progressive changes in castrated mice of the ce strain.

CARCINOMAS OF THE ADRENAL CORTEX

A. Frequency.—No cortical tumors were observed in adrenals from the intact group. In the castrate series the first ones were observed in the 7 month age group (see Fig. 3). Of the 19 mice from 7 to 12 months of age inclusive, 15 had carcinomas of the adrenal cortex. All of the 5 mice killed at 12 months of age had cortical neoplasms. Of the 15 mice with adrenal carcinomas 4 had bilateral growths. Bilateral occurrence did not increase greatly with advancing age. It appeared in animals 9, 10, and 12 months of age. P2269, a mouse with a large adrenal growth at 12 months of age (see below), had a tumor in only one adrenal.

B. Size.—To obtain a general idea of the size of these cortical tumors they were classified in 3 groups according to their dimensions: (a) microscopic to 0.5 cm. in diameter (small); (b) 0.5 to 1.5 cm. (medium); and (c) greater than 1.5 cm. (large). If 2 tumors were present in 1 animal, they were considered as one. On this basis it was found that 12 mice had small, 2 medium, and 1 a large amount of adrenal tumor tissue. A size classification was attempted in an effort to see if the amount of tumor tissue had any relation to the degree of accessory sex organ development. As will be seen later, a relationship does seem to exist. The individual numbers and their respective tumor size are listed in Table II.

C. Appearance.—The gross and microscopic appearance was similar to that described in ovariectomized females of the ce strain (23), and will not be further discussed here (Figs. 2 to 6 inclusive).

D. Metastases.—No metastases were found.

OBSERVATIONS ON THE SEX ORGANS

Seminal vesicles and prostate.—The accessory sex glands, seminal vesicles and prostates of the 33 control mice were observed macroscopically and then sectioned and studied microscopically. At 1 month the seminal vesicles were of medium size, with some secretion (Table II). At 2 months and later they were large and distended with secretion.

The same method of study was used for the 39 castrated males. Enlargement of the accessory sex glands in the castrated series was slight before the 11th month. The seminal vesicles and prostate were definitely enlarged and secretion was evident in P2270 (Figs. 12 and 16) and P2269, with medium-large and large cortical tumors, respectively. In 3 mice, P2217, P2213, and P2272, squamous metaplasia of the prostatic epithelium was observed (Figs. 13 and 15). This is a condition that has been produced in male animals by the use of estrogenic substances [2] (3, 4, 10). Albert (1) found that the squamous metaplasia caused by folliculoid substances is not prevented by testosterone. However, probably not much androgenic hormone was present in these particular mice as shown later by observations on the submaxillary gland. Also, no mouse in this series with secretion in the seminal vesicles had squamous metaplasia of the prostatic epithelium. In a number of instances, especially in mice with squamous metaplasia of the prostate, there was a great increase in hyaline connective tissue stroma of the gland. An example of this is shown in Fig. 13. Burrows (3) found that this condition and metaplasia of the prostatic epithelium accompanied estrogen injection. Hyaline connective tissue and seminal vesicle secretion were both present in one castrate male.

Testes.—The testes of 30 control males were observed microscopically. A few spermatozoa were present in the tubules of males killed at 1 month, and beyond this age spermatogenesis was active and normal. No abnormalities of the testes were recorded.

Mammary glands.—The mammary glands of intact male mice were fixed with the skin and examined with a dissecting microscope. All were either rudimentary or absent. This is in line with our observations on males of the dilute brown strain (21). The rudimentary glands of the males at 1 month of age were the most easily found of the entire series. Gardner and his associates (9) noted that the mammary rudiments removed from male mice from 12 to 15 days of age were as well developed as those from

[2] Males of strain C57 black will show this response when receiving estradiol benzoate at a level of 16.6 μgm. weekly, while male mice of the CBA strain rarely show such a response to continuous estrogenic treatment at this level. GARDNER, W. U., Cancer Research, **1**:355. 1941.

TABLE I: OBSERVATIONS ON NONBREEDING MALE MICE OF THE CE STRAIN

Age, months	Number examined	Adrenal: Number of tumors	Adrenal: Foci type A cells	Adrenal: X zone	Submaxillary gland	Testes	Accessory sex glands	Mammary glands
1	4	0	absent	narrow	♂-type	normal	medium size, some secretion	Rudimentary or absent
2	4	0	present	hyperemic	"	"	large size, normal secretion	"
3	5	0	"	"	"	"	"	"
4	3	0	"	absent	"	"	"	"
5	1	0	"	"	"	"	"	"
6	1	0	"	"	"	"	"	"
7	3	0	"	"	"	"	"	"
8	2	0	"	"	"	"	"	"
9	4	0	"	"	"	"	"	"
10	1	0	"	"	"	"	"	"
11	4	0	"	"	"	"	"	"
12	1	0	"	"	"	"	"	"

TABLE II: OBSERVATIONS ON CASTRATED MICE OF THE CE STRAIN 1 TO 12 MONTHS OF AGE, INCLUSIVE

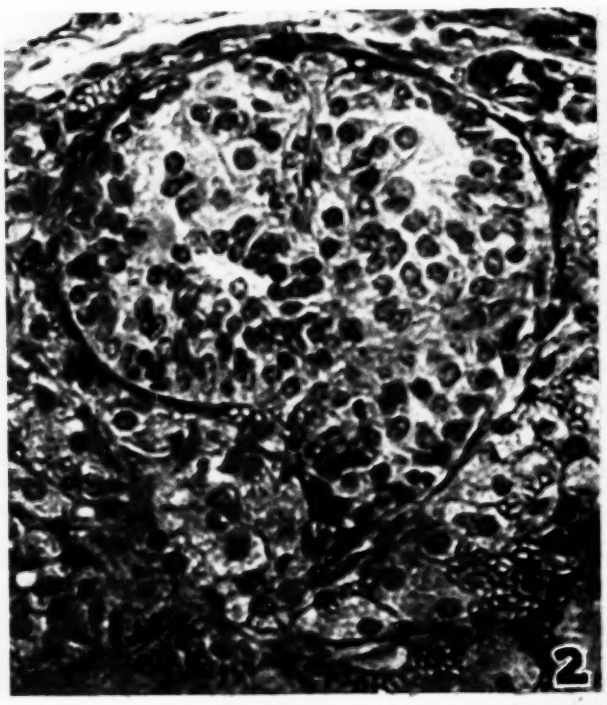
OBSERVATIONS ON MICE WITHOUT ADRENAL CORTICAL CARCINOMAS

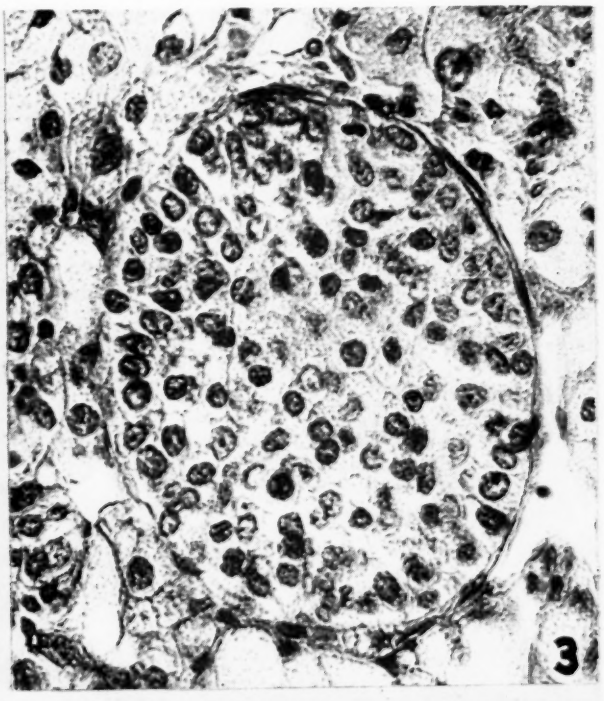
Age, months	Number observed	Adrenals: Foci type A cells	Adrenals: Foci type B cells	Adrenals: X zone	Accessory sex glands	Submaxillary gland	Mammary glands
1	4	absent	absent	present	small	♀-type	Rudimentary or absent
2	4	present	"	"	"	"	"
3	2	"	"	irregular	"	"	"
4	4	"	"	"	"	"	"
5	1	"	"	"	"	"	"
6	5	"	present	"	"	"	"
9	3	"	"	absent	medium	intermediate	"
11	1	"	"	"	"	"	"

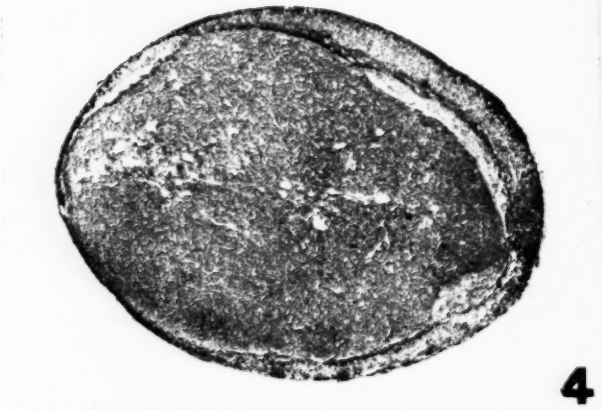
OBSERVATIONS ON MICE WITH ADRENAL CORTICAL CARCINOMAS

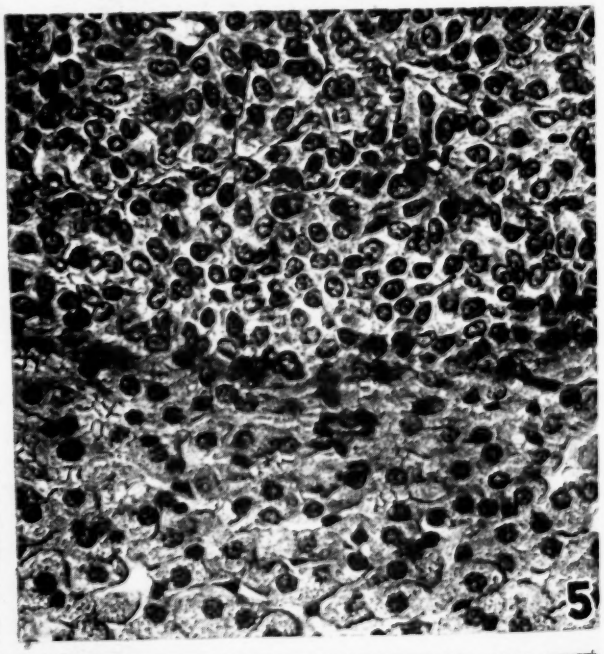
	Mouse number	Adrenal: Number of carcinoma	Adrenal: Tumor size *	Submaxillary gland	Accessory sex glands: Size	Accessory sex glands: Stratified squamous epithelium	Accessory sex glands: Secretion in seminal vesicles	Mammary glands: Duct length	Mammary glands: End buds
7	P2263	1	small	intermediate	slight enlargement	none	absent	rudimentary	absent
7	P2264	1	"	"	"	"	"	"	"
8	P2258	1	"	♂-type	"	"	"	"	"
9	P2265	1	"	intermediate	"	"	"	long	"
9	P2315	2	"	"	"	"	"	rudimentary	"
9	P2693	1	"	" †	"	"	"	"	"
10	P2157	2	"	" †	"	"	"	"	"
10	P2184	1	"	" †	"	"	"	short	"
10	P2185	1	"	"	"	"	"	rudimentary	"
10	P2158	1	"	" †	"	"	"	"	"
12	P2270	2	medium	♂-type	medium to large	"	present	medium	"
12	P2217	1	small	intermediate †	"	present	absent	"	present
12	P2213	1	"	" †	"	"	"	long	absent
12	P2269	1	large	♂-type	"	none	present	medium	present
12	P2272	2	medium	intermediate †	"	present	absent	long	absent

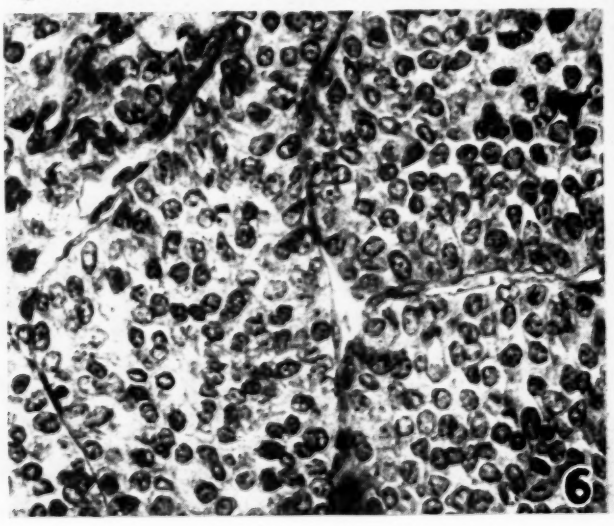
* Microscopic to 0.5 cm. in diameter = small.
0.5 to 1.5 cm. in diameter = medium.
Greater than 1.5 cm. in diameter = large.
† Almost the same as female type.

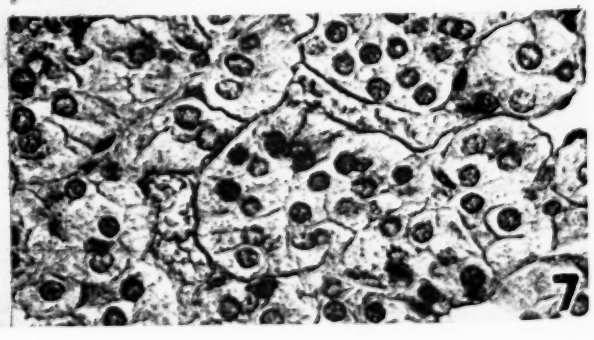












FIGS. 2-7

normal males under 100 days, over 200 days, and over 700 days of age. Lewis and Turner (11) examined 164 normal male mice (15 to 25 gm.) and found no rudiments consisting of more than a few primary ducts with perhaps a few short, wide stubs. These glands varied in length from 0.4 to 5.0 mm. with an average for thoracic, sternal, and abdominal pairs [3] of 1.2 mm. The rudimentary glands in the males of the ce strain were separate from the nipples.[4]

Recently it has been observed (12) that males of a strain of Swiss mice had a "high proportion of fairly well developed mammary ducts." This is in contrast to the usual rudimentary mammary gland of the male mouse. Male mice of the ce strain have the rudimentary type of mamma.

The mammary glands of 39 castrated males were examined. Unusual extension of mammary ducts over that of intact males was found for the first time in animals 9 months of age. This is slightly later than the time at which pronounced duct extension was first seen in the ovariectomized females. This is also near the time when extensive arborization of the mammary ducts was observed in the virgin female group. Extensive duct extension did not occur in any castrated male that did not have a carcinoma of the adrenal cortex, and conversely it did occur in all mice with medium or large size tumors.

P2217, 12 months of age, with a small tumor; and P2269, 12 months of age, with a large tumor both had mammary ducts with terminal end buds, a type of growth correlated with early periods of estrus in female mice. Terminal end buds, indicating rapid mammary growth, were found in male mice injected with theelin (9). No localized adenomatous growths such as have been reported for male mice of certain strains following estrogen injection, were noted. This finding may, as in the case of the female, be correlated with low mammary tumor incidence in strain ce, as association of these adenomatous types of growth with strains having high mammary tumor incidence and not with strains having a low mammary tumor incidence has been reported (2, 5). Nodules were found at a later age in castrated dilute brown mice (21). This strain has a high incidence of mammary tumors in both virgin and breeding females.

Growth of the mammary ducts was not uniform from gland to gland within individual mice. When the gland pairs were numbered cephalad to caudad the most frequently developed were the third, second, first, fourth, and finally the fifth, without any indication of duct increase. Glands on the right and left sides were almost equally developed. This series was not the order of development in castrated males of the dilute brown series (21). No development of the alveolar system was found.

As in ovariectomized female mice of strain ce (23), it is not easy to ascribe the growth of the mammary glands to specific hormones. Estrogenic hormones might have caused the growth, as they have proved their effectiveness experimentally in the hands of a large number of workers. Lewis and Turner (11) found that testosterone propionate did not influence the gland rudiments in less than 25 days. One milligram of dehydroandrosterone in oil was effective in 6 days. Certain pituitary extracts were also effective in causing growth of the mammary rudiments of castrated male mice.

Pituitary.—The pituitary glands of the gonadectomized and of the control mice were sectioned and observed. No striking differences [5] between any groups have been noted during the ages covered in this report. The question whether hyperfunction of the adrenal cortex or a disturbance arising in the pituitary is primary in the adrenogenital syndrome has not been

[3] The second, third, and fourth pairs numbering cephalad to caudad.

[4] Turner and Gomez (16) state that in the 17 day fetus of the mouse, in the anlage of the gland, the mammary bud can be shown to be separated from the primary sprout. Indications are that the rudiment of the bud undergoes cornification and desquamation until in the 18 to 19 day fetus the rudiment of the bud is seldom observed. The sprout appears as an isolated structure in the corium. A definite lumen is formed and secondary sprouts are developed. At the beginning of extrauterine life the proximal end of the primary sprout is still uncanalized. The other end of the sprout and the rudimentary secondary sprouts possess lumina slightly more advanced in development than in females of corresponding ages. At this stage the mammary duct ceases to grow and remains rudimentary throughout normal life.

[5] No special pituitary staining technic was used.

DESCRIPTION OF FIGURES 2 TO 7

FIG. 2.—Small tumor in adrenal cortex of mouse P2315, 9 months old; capsule of gland at top. Mag. × 400.

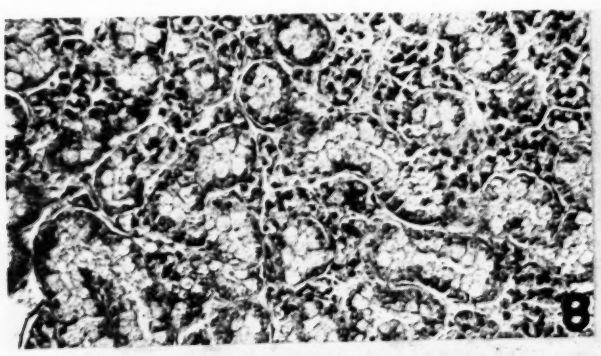
FIG. 3.—Small tumor in adrenal cortex of P2263, 7 months old. Note enlarged cells around tumor. Mag. × 500.

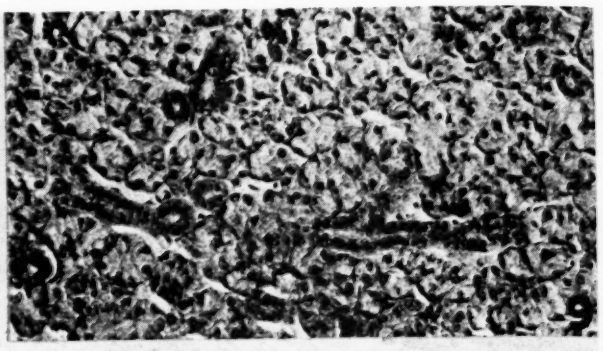
FIG. 4.—Adrenal cortical carcinoma in adrenal of P2315, 9 months old. Note adrenal cortex and adrenal medulla, especially at right. Mag. × 20.

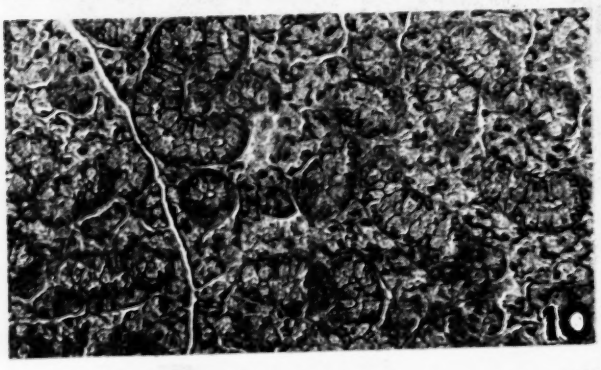
FIG. 5.—Adrenal carcinoma (above) displacing adrenal cortex. Mag. × 400.

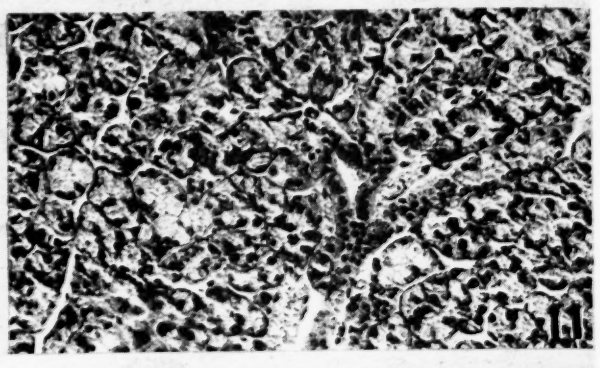
FIG. 6.—Showing lobulation, a frequent characteristic in adrenal cortical carcinomas, in a small adrenal tumor. P2315. Mag. × 400.

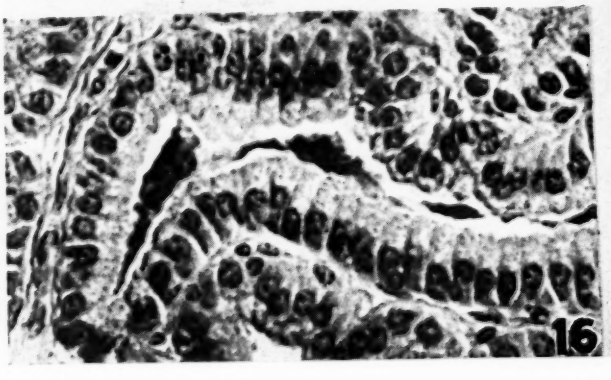
FIG. 7.—Cells of adrenal medulla in P2315 for comparison with those of adrenal cortical tumor in same animal (Figs. 5 and 6). Mag. × 400.

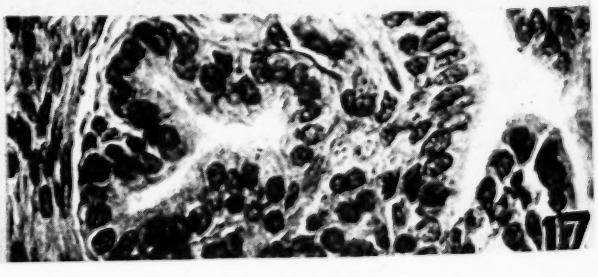












FIGS. 8-17

satisfactorily answered (18). Our observations do not answer this question, but at least we may say that in our series, and under our method of observation, the changes in the adrenal cortex were the first to be obvious.

Submaxillary gland.—In the normal ce male the epithelial lining of the terminal tubules was made up of tall columnar cells. Beginning at 1 month these tubules could be easily differentiated from those in the female mice. The nuclei were at the base of the cells and were large and round. In these respects strain ce is in definite contrast to strain dba and strain C57 black where, in young animals of this age, structural differences between males and females do not exist (6). At ages greater than 1 month the tubule cells remained tall columnar. The nuclei gradually became shrunken, irregular in outline, and more or less flattened at the base of the cell. As age advanced the intralobular ducts occupied more and more space within the gland as a whole (Fig. 8). This probably accounts for the fact that the gland became lighter in color, when examined in the gross. It would account for the other gross observation, that the submaxillary gland increased in size with an increase in age provided the glandular increase was not made at the expense of the acinar tissue. As age advanced there was a slight increase in the size of the lumina between the tall columnar cells.

The findings for the submaxillary glands of the 39 castrated males are tabulated in Table II. For the first few months the glands were very similar to those of the young ovariectomized females, and also similar to those of normal females of the ce strain (Fig. 9). At 6 months, and through 7 and 8 months, there was a very slight alteration to the male type of gland. The first evidence of change was that the cells of some of the terminal tubules had become more columnar and the nuclei had assumed a basal position. At 9 months this process had progressed further. It is interesting that at this age mouse P2688 is the most male type, and yet it had no adrenal cortical carcinoma. It seems possible that nodules of type A and type B cells in the adrenals are sufficient to change the type of the submaxillary gland. This is not unexpected, since cells of these types apparently secrete hormones that stimulate accessory sex glands in the dilute brown series (7, 19, 21). At 11 and 12 months the terminal tubules of the submaxillary glands had tall columnar cells with nuclei flattened at their bases. The tubules occupied a high percentage of the total area of the gland (Fig. 10). P2269 was similar in this respect to an intact male of 7 months. Considerable variation in the submaxillary glands occurred in the castrated males following the first evidence of change from the type observed during the first few months (Fig. 11). P2217, for example, was only slightly changed from the early type, although the animal was 12 months of age. The cortical tumor in the adrenal of this animal was small.

DISCUSSION

This report shows that there is a simple way to produce carcinomas of the adrenal cortex in male mice. It involves: (a) selection of a suitable strain of mice, and (b) removal of the gonads at an early age. The strain used is probably of great importance, since these neoplasms were not observed in dba males castrated at birth or later, and maintained to advanced ages (21). If castrated males follow closely the pattern set by ovariectomized females, adrenal cortical carcinomas would also be difficult to obtain by castration of C57 black and C3H males (20). It is not known how important it is that the animal be castrated at a very early age, as in this experiment. Nodular hyperplasia of the adrenal cortex was observed in mice of the dba strain when they were castrated as late as 6 months after birth (21). Gardner (8) obtained tumors of the adrenal cortex in females of strain NH that had been ovariectomized at 43 to 65 days of age. It has been pointed out that hormone imbalance has been found an important factor in the

DESCRIPTION OF FIGURES 8 TO 17

FIG. 8.—Typical submaxillary gland in male mouse 11 months old. Mag. × 150.

FIG. 9.—Typical submaxillary gland in female mouse 12 months old. Mag. × 150.

FIG. 10.—Submaxillary gland of P2270, 12 months old; male type in castrated male with adrenal cortical carcinoma. Mag. × 150.

FIG. 11.—Submaxillary gland of female type in castrated male P2213, 12 months old, with adrenal cortical carcinoma. Mag. × 150.

FIG. 12.—Section through accessory sex organs of masculinized castrated male P2270, 12 months old, with adrenal cortical carcinoma. Mag. × 20.

FIG. 13.—Section through accessory sex organs of feminized castrate male P2213, 12 months old, with adrenal cortical carcinoma. Mag. × 20.

FIG. 14.—Epithelium of prostate of castrated male P2270. Mag. × 500.

FIG. 15.—Squamous metaplasia of epthelium of prostate in feminized castrated male P2213. Mag. × 150.

FIG. 16.—Tall columnar epithelium with secretion in seminal vesicle of castrated male P2270. Mag. × 500.

FIG. 17.—Low epithelium and no secretion in seminal vesicle of feminized castrated male P2213. Mag. × 500.

causation of a number of different types of neoplasm (22).

A comparison of the findings for gonadectomized males and females of the ce strain (23) shows many similarities and a few important differences. The sexes are similar, as far as the adrenal is concerned, in that the same general focal changes appear in the cortex before the occurrence of adrenal carcinoma, and similar again in that cortical carcinomas occur in both males and females. There is also evidence that these adrenal neoplasms are associated in both with increase in the size and activity of accessory sex organs. An interesting difference is the general observation that on a basis of the number of tumors present, and on their size, the females were always well in advance of the males at any given age. Thus a difference in the susceptibility of males and females seems to exist. For the two sexes taken together, the period between 6 and 12 months inclusive is the tumor period. In this period 20 ovariectomized females were examined, and all were found to have adrenal tumors. In the same age period 15 of 24 castrated males were thus affected. Furthermore, 11 of the females and only 4 of the males had bilateral growths.

As to size (Table II), 11 females had small, 6 medium, and 4 large amounts of tumor tissue, whereas 12 males had small, 2 medium, and 1 a large amount. In this regard, it is interesting that adrenal tumors have been reported to occur more frequently in female than in male children; Young (25) found that of 31 cases in children, 26 were females and 5 were males. Taylor and Wiseman (15) collected 73 cases of "adrenal cortical malignant disease" from the literature. Thirty-seven of these were in persons over 15 years old, and 36 were in children. Of the 36 children, 25 were females and 11 were males. The lesion occurred 21 times on the left side, 9 times on the right, was bilateral in 1 case, and not recorded in 5 cases. No division as to sex and side was made for the older group.

SUMMARY

Observations have been made on 33 intact and 39 castrated male mice of the ce strain. Data were recorded at monthly intervals on animals ranging from 1 to 13 months in age.

Adrenal cortical carcinomas were observed in 15 of 19 castrated mice killed at 7 to 12 months of age inclusive. In 4 cases the tumors were bilateral, so that the 15 mice carried 19 tumors. No such growths were found in 15 intact males killed at the same ages.

The adrenal tumors were preceded by certain definite changes in the cortex, which involved pronounced focal increase in subcapsular cells and later, localized enlargement of cells.

The condition of the accessory sex organs in the castrated mice indicated that they were being subjected to: (a) an estrogenic influence in some individuals, and (b) to an androgenic influence in others. There was some evidence that the difference between individuals was in some cases due only to a variation in the proportion of estrogens and androgens, both possibly being present at the same time.

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Histological Study of Adrenal Cortical Tumors in Gonadectomized Mice of the ce Strain*†

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(Received for publication October 14, 1944)

It has recently been reported from this laboratory that carcinoma of the adrenal cortex occurred in a high percentage of cases in extreme dilution strain ce mice that were gonadectomized at 2 days of age (3). The material collected from these animals is especially valuable because it gives a chance to study and detect the very early changes that precede and lead to the formation of rare tumors of this type. Therefore, in this histopathological study special emphasis will be given to the description of these early changes, which are unique in this strain and may justifiably be considered as significant steps leading to tumor formation.

MATERIAL AND METHOD

A total number of 131 gonadectomized mice were observed, 70 spayed females and 61 castrated males. Gonadectomy was performed at 2 days of age. The method of removing the ovaries has been described previously (1, 2). The testes were approached through a small midventral incision, and the epididymides and a short part of each vas deferens were also removed.

Groups of experimental animals were killed at monthly intervals, and in every case a careful search was made for gonadal regeneration. In the earlier age groups both adrenals were serially sectioned; in the older age groups when the adrenal tumors were very large serial sectioning seemed unnecessary and only a few sections were cut.

Twenty normal virgin females and 23 males were used as controls. Both adrenals of all these animals were serially sectioned. No adrenal tumors occurred in any of them.

Observation on the Adrenals of Spayed Females

At 4 months.—The earliest abnormal areas, which were unique in the ce strain of mice, were found in the adrenals of animals killed at 4 months of age. The significance of these areas became evident only because one was able to observe what followed them rather than the way they were at this stage.

They were composed of a small group of cells in follicular or arch-like arrangement, which occasionally contained a lumen, and were situated immediately below the connective tissue capsule. The cytoplasm of these cells formed a syncytial protoplasmic network, in some cases filling the lumen. The nuclei that were scattered throughout this network were oval in shape, contained very finely distributed chromatin granules, and usually one prominent compound nucleolus. The areas were surrounded by circularly arranged connective tissue fibers (Fig. 1). The whole structure resembled somewhat the seminiferous tubules of a cryptorchid testis in which only Sertoli cells are present in a protoplasmic syncytium.

The adrenals of 3 out of 4 mice belonging to this age group contained such areas, and in each of 2 glands 2 were present.

At 5 months.—Only 1 animal was killed at this age and a similar abnormal area was present in one adrenal.

At 6 months.—Five animals represented this age group. The next series of changes was perhaps best represented by one adrenal of mouse 2088. There were now several groups of round areas in close proximity, composed of cells in syncytial arrangement, and their presence caused a slight elevation on the surface of the adrenal. At the base of some of these areas large polyhedral cells were present arranged in a stratified layer. They had large oval nuclei containing chromatin granules of medium size and several nucleoli. A small amount of slightly basophilic cytoplasm surrounded each nucleus. These cells infiltrated about half way into the fascicular zone and destroyed its cell components. Similar cells formed the bulk of the large tumors that occurred in the adrenals of the older animals. The fact that at their early appearance these cells were in close association with areas composed of syncytial cells perhaps justifies the postulate that the latter areas were precancerous lesions.

The other adrenal of this animal (2088) contained

* This work has been aided by grants from the Commonwealth Fund, The Anna Fuller Fund, The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund, the National Advisory Cancer Council, and The Rockefeller Foundation.

† The histological data were collected from mice gonadectomized by George W. Woolley, Ph. D., in preparation for further studies, which will be reported by him.

a small precancerous area, which caused a slight bulging of the capsule.

In one adrenal of each of 2 other animals (2086 and 2175) small cancerous areas were present in irregular nodular arrangement. In the fourth animal of this group the neoplastic area in one adrenal was considerably larger, projecting about half way into the medulla. The tumor consisted entirely of large polyhedral cells and showed many mitotic figures. It was not encapsulated, although the surrounding cell columns and sinusoids, which were compressed by the expanding growth at some places, gave this impression. The other adrenal did not show precancerous or cancerous changes.

Both adrenals of the fifth mouse in this group were normal.

At 7 months.—Four animals were killed at 7 months of age. In one adrenal of mouse 2252 the neoplastic area involved the whole depth of the cortex at that focus; in the other, both precancerous and cancerous areas were present in close proximity. Enlarged cells, some in mitotic division, were present between the fibers of the capsule, causing a slight elevation of the gland surface.

One adrenal of mouse 2259 was normal. In the other, which was somewhat enlarged, the cancerous area occupied more than half of the gland. In this, as in all the larger tumors, the nuclei were more pleomorphic, the cytoplasm was slightly basophilic and small in amount, and the cell outlines were indistinct. Mitotic figures were numerous. A rich network of capillaries provided a good blood supply. The stroma was very scanty.

Small carcinomatous areas were present unilaterally in the adrenals of mice 2260 and 2250 while the opposite glands were normal.

At 8 months.—One animal was killed in this age group (2257). Both adrenals were carcinomatous, and one of them was greatly enlarged.

At 9 months.—Two mice represented this age group (2686 and 2689). In 1 a tumor of medium size was present in one adrenal, while the opposite gland was tumor-free. In the other animal (2689) one adrenal was considerably enlarged by tumor. In the opposite gland a small tumor was present near the capsule.

In the adrenals of both these animals a new type of tumor cell occurred, which seemed to have originated between the fibers of the capsule. These cells were small, cuboidal in shape, and arranged in rows. They had large, dark-staining, oval nuclei with evenly distributed, coarse chromatin granules. A small amount of slightly basophilic cytoplasm surrounded each nucleus. These cells resembled the follicular cells of young ovarian follicles.

Older Age Groups

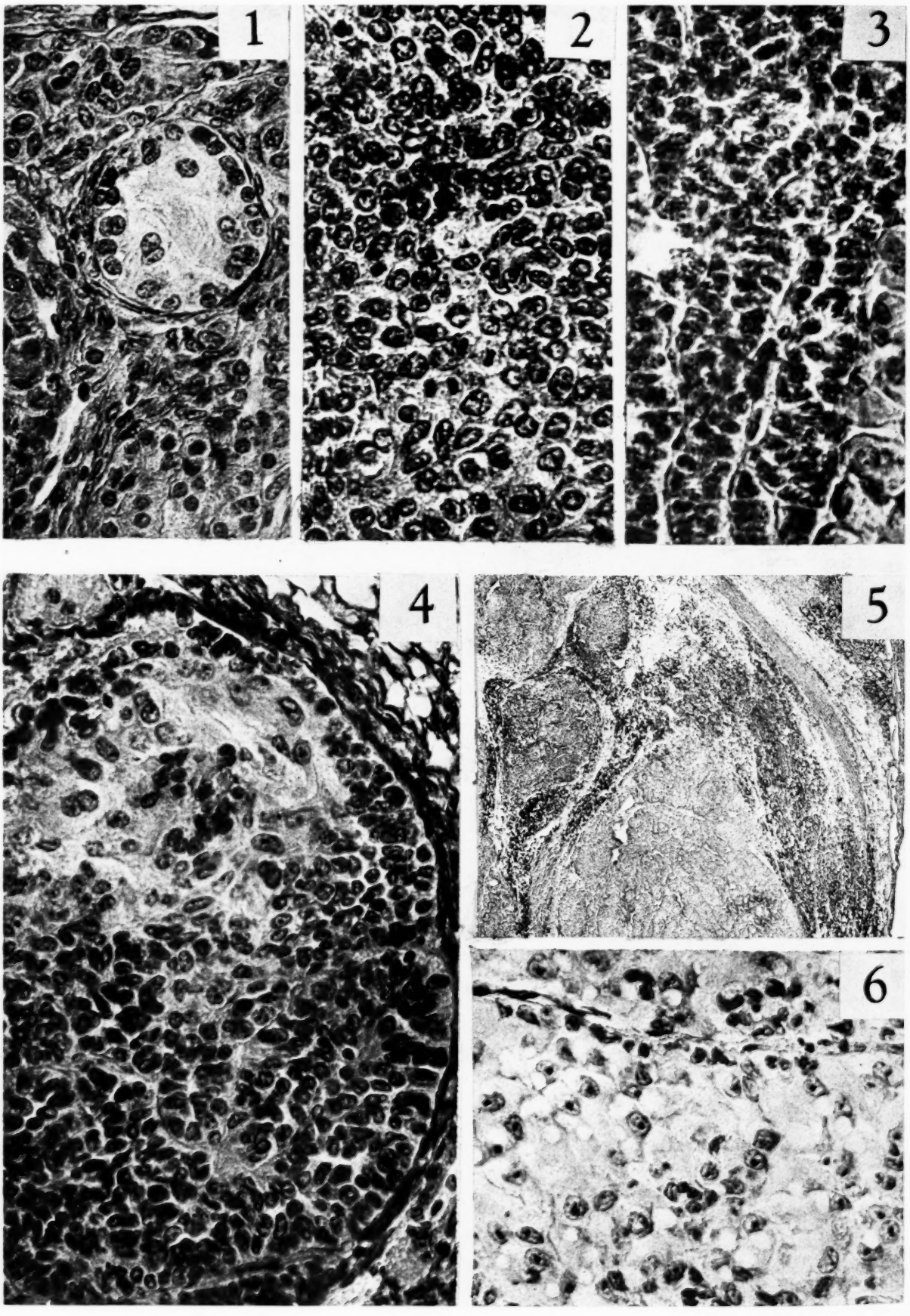
From this age on, description of the tumors of individual age groups seems unnecessary. The cells already described were present in all, differing only in proportion. In almost all instances the second (cuboidal) cell type occurred only when a tumor of considerable size formed by the first (polyhedral) cell type was already present. However, there were a few exceptions. In one adrenal of mouse 2273 (11 months) rows of cuboidal cells were present between the fibers of the capsule at a wedge-shaped area, which caused a slight elevation of the gland surface. At the inner aspect of this a small follicle-like area was present in which the central part was filled with nuclei scattered in a protoplasmic network (precancerous area), while at the periphery polyhedral cells were present in irregular arrangement. In the other adrenal a medium-sized tumor composed of the polyhedral type of cell was present.

In most of the large tumors the bulk of the tissue was formed by diffusely arranged polyhedral cells which, judging from the number of mitotic figures, multiplied more rapidly (Fig. 2). The cuboidal cell type was usually arranged in rows and cords showing some degree of attempted gland formation (Fig. 3). Although usually these cells were more prominent near the periphery, they were also found intermingled with the diffusely arranged polyhedral cells. Scattered among both types of tumor cells, giant cells possessing yellow pigmented cytoplasm and often multiple nuclei were found in many tumors in varying numbers. Blood vessels were numerous and consisted mainly of capillaries and endothelium-lined spaces between groups of tumor cells. In smaller carcinomas the stroma was very scanty, forming only a delicate framework (Fig. 4). After the capsule of the gland was invaded, larger trabeculae projected into some of the larger tumors, dividing them into irregular lobules (Fig. 5).

In some cases the syncytial type of cell, which was described as forming precancerous areas, was present near the periphery of cancerous areas (Fig. 4). In one large neoplasm this cell type evidently persisted and formed a considerable part of the tumor. The syncytial cytoplasm of these cells contained lipid globules (Fig. 6).

Many of the large tumors had broken through the adrenal capsule; however, this was usually present around a small part of the gland. Beneath it remnants of normal cortical tissue and narrow compressed zones of medullary cell cords were always present. In the large tumors the central areas often showed hemorrhage, necrosis, and occasionally calcification.

While the normal size of a mouse adrenal is about



FIGS. 1-6

1 × 2 mm. in diameter, several of the cancerous glands reached 15 × 20 mm. and were easily detectable in the live animal by palpation.

Two animals that showed attempted ovarian regeneration should be mentioned individually. Mouse 1147 (killed at 13 months) showed at autopsy a dense nodule in the adipose tissue of the mesosalpinx and microscopic examination gave proof of the presence of ovarian tissue. Several primary and anovular follicles, areas of luteinized cells, and small cysts lined by ciliated columnar epithelium were present. The bulk of the nodule was composed of atypical cells forming an ovarian carcinoma. This mouse also had a large adrenal cortical carcinoma. Structurally the ovarian and the adrenal carcinomas were strikingly similar. A medium-sized tumor was found in the ovarian region of mouse 2418 (killed at 15 months). Microscopic sections did not reveal any normal ovarian tissue and there was only gross evidence of an ovarian origin. This tumor also resembled structurally the adrenal cortical carcinomas.

An interesting anatomical difference existed between other strains and the ce strain concerning the position of the adrenal glands. While in other strains there is a distance of about 2 mm. between the inner aspect of the cephalic pole of the kidneys and the adrenals, in the ce strain the surfaces of these 2 organs are in direct proximity. In spite of this the kidney was never invaded by the adrenal tumors.

Metastases to the lungs were observed in microscopic sections in 8 instances. As the lungs were sectioned only when suspicious nodules were noted upon gross examination some of the smaller metastases may well have escaped detection.

To summarize the changes that seemed to occur in 3 main steps: (a) The first step was the appearance of small groups of syncytial cells near the capsule, characterized by oval nuclei scattered in a protoplasmic network. The nucleus contained very fine chromatin granules and usually one prominent compound nucleolus. These areas formed round follicles, which occasionally contained a lumen and were surrounded by circularly arranged connective tissue fibers. Since in later abnormal development these areas played a prominent part they were considered as precancerous regions. They occurred first in the adrenals of 4 month old animals, but were also found later at the periphery of already cancerous areas. (b) The second step was the appearance of atypical polyhedral cells, first at the base of the follicles that formed the precancerous regions. The nuclei at first were mostly oval—later pleomorphic, and contained medium-sized chromatin granules and several nucleoli. The cytoplasm was slightly basophilic and small in amount, there was a lack of polarity, and from their earliest appearance these cells showed unusual mitotic activity. In diffuse masses they projected first toward the center of the gland and later outward, breaking through the capsule. (c) The third step was the appearance of rows of cells that seemed to originate between the fibers of the adrenal capsule in animals in which tumors formed by polyhedral cells were already present. These cells were cuboidal in shape, and had oval dark-staining nuclei that contained coarse chromatin granules. They were arranged in parallel rows or cords showing an attempted gland formation. They usually occupied a peripheral position, but in the larger tumors they were also found intermingled with polyhedral cells. They were rarely seen in the process of mitotic division.

Observations on the Adrenals of Castrated Males

At 4 months.—The adrenals of 3 animals were examined at this age. In one adrenal of mouse 2082 a small round area was present, composed of cells in syncytial arrangement. The cells were similar to those found in the adrenals of several of the ovariectomized females of this age group.

At 5 months.—One animal was examined at this age. In one adrenal a small, completely encapsulated, benign tumor was present immediately below the capsule. It was composed of polygonal cells with large, round nuclei surrounded by small amount of cytoplasm.

At 6 months.—Five animals were sacrificed at this age; none of the adrenals contained tumors. Small areas composed of cells in syncytial arrangement were present below the capsule in the adrenals of 2 mice unilaterally.

At 7 months.—Two mice were killed at this age. Unilateral cortical tumors were present in 2 of them. In both adrenals the neoplastic areas were narrow, but extended from the capsule to the medulla. The nuclei of the tumor cells were mostly oval, though showing some variation in shape and size, and contained finely distributed chromatin granules and sev-

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Syncytial cells in follicular arrangement. Mag. × 450.

Fig. 2.—Diffusely arranged polyhedral cells. Mag. × 600.

Fig. 3.—Cuboidal cells arranged in rows. Mag. × 600.

Fig. 4.—Syncytial cells and polyhedral cells in a tumor of medium size. Note scant stroma. Mag. × 450.

Fig. 5.—Irregular lobular architecture of a large tumor. Mag. × 75.

Fig. 6.—Syncytial cells containing lipid globules. Mag. × 450.

eral nucleoli; the cytoplasm was scanty and slightly basophilic. The cells were polygonal, showed a lack of polarity, and several were in the process of mitotic division. The tumors were not encapsulated and were considered carcinomas; one was surrounded by an area of hypertrophied cells and a slight elevation of the gland surface was evident.

At 8 months.—One animal with a small cortical carcinoma in one adrenal represented this age group.

At 9 months.—Five mice were killed at this age. In 2 animals cortical carcinomas were present unilaterally, and in 1 bilaterally. In mouse 2693 about half of the gland had been destroyed and replaced by tumor cells. The carcinomatous adrenal of mouse 2263 was about 3 times as large as the normal gland. One adrenal of mouse 2315 was somewhat enlarged by the carcinoma, while the opposite gland contained a very small tumor. All these neoplastic areas were composed of large, irregularly arranged, polyhedral cells similar to those that formed the tumors of ovariectomized females.

At 10 months.—Four mice represented this age group. Small cortical carcinomas were present in 2 animals bilaterally and in 2 unilaterally.

At 11 months.—A small cortical tumor was found in one adrenal of mouse 2266, which was killed at this age.

At 12 months.—Four animals were killed at this age. Adrenal tumors occurred in 3 of them unilaterally and in one bilaterally. All the glands containing neoplastic areas were considerably enlarged. Up to now the tumors had been composed of the large polygonal cells, but from this time on small cuboidal cells with deeply staining oval nuclei were present in many of the growths. As in the ovariectomized females, this second cell type appeared first between and beneath the fibers of the capsule of those adrenals in which tumors of the polygonal cell type were already present.

Older age groups.—In most of the neoplasms of the older age groups the large polygonal cell predominated and, judging from the number of mitotic figures, grew more rapidly. The small cuboidal cells showed a tendency to form rows and cords and to surround areas composed of giant cells with yellow pigmented cytoplasm. These "yellow giant cells" were consistently observed in the adrenals of castrated males, but did not seem to have any active part in tumor formation. Although they were present in the adrenals of gonadectomized females they were more numerous in the adrenals of the males.

In one adrenal of mouse 2529 (23 months) a small tumor was composed exclusively of cuboidal cells and yellow giant cells. In the opposite adrenal a large growth, composed mostly of large polygonal cells, was present; an area composed of nuclei scattered throughout a syncytium and similar to that shown in Fig. 6, was also present in this tumor.

Lung metastases were not noted in any of the castrated males.

In general the adrenal tumors of the gonadectomized females and males were fairly similar in structure. Points of difference were: (a) the cuboidal tumor cells first appeared at a later age in the males, 9 months in females, 12 months in males; (b) there were more "yellow giant cells" present in the tumors of the males; (c) judging from the size of the tumors those in the males grew more slowly.

The total percentage of adrenal tumors was 72.54 in the males and 91.90 in the females. There were 54.05 per cent of unilateral tumors in the males, and 31.03 per cent in the females. Bilateral tumors occurred in 45.94 per cent of the males, and 68.96 per cent of the females. Only animals more than 3 months old are included in these figures. Tables I and II give the results in tabulated form.

DISCUSSION [1]

Carcinomas of the adrenal cortex are rare, and their occurrence in so many of the gonadectomized animals of the extreme dilution strain ce makes logical the conclusion that early removal of the gonads was responsible for their appearance. As it is recognized that some functional relationship exists between the adrenal cortex and the gonads, it is possible that the initial growth leading finally to neoplasia was an effort on the part of the cortex to compensate for the absent gonads. Whatever the initial reason, it is evidently characteristic of the extreme dilution strain ce, but not of other strains tested, dba and C57 black (4), where gonadectomy did not lead consistently to the occurrence of cortical carcinomas. There are 2 unique factors in the ce strain that might have had an important bearing on the occurrence of cortical tumors: the peculiar anatomical position of the adrenals, and a constitutional hypogenitalism.

It has been mentioned previously that the adrenals of the ce strain are in direct proximity to the kidneys, while in the dba and C57 black strains they are about 2 mm. farther cephalad. In embryonic development the adrenals originate from the celomic mesoderm in the region of the genital ridge; in the ce mice they evidently remain closer to their original position, perhaps as an outcome of retarded development, for which histological proof was noted. At 1 month of age the cortex was not yet completely separated from the medulla, but penetrated it partially with finger-like projections.

[1] The hormonal effects of the adrenal cortical tumors will be discussed in a separate paper by Dr. George W. Woolley.

As evidence for the second factor, namely, the constitutional hypogenitalism, the following observations are advanced: Data collected on the breeding behavior of ce mice showed that the females seldom had their first litters before the age of 4 or 5 months, that they stopped breeding earlier than females of some other strains, and that complete sterility frequently existed in both sexes. Histological examination of ovaries often showed a scarcity of ova. An additional aberration from normal was the fact that in many ovaries follicle-like structures were found, which contained a syncytial network with scattered nuclei resembling Sertoli cells of a cryptorchid testis. This was seen in both breeding and nonbreeding females. Spontaneous gonadal tumors occurred in numerous instances, particularly in the ovary. Some of these ovarian growths structurally resembled the adrenal cortical carcinomas of gonadectomized animals. It has been mentioned previously that a similarity in structure existed also between the adrenal cortical neoplasms and the ovarian tumors that occurred in the regenerated ovaries of 2 of the gonadectomized females.

There are 2 interesting questions about the histogenesis of these tumors. (a) What structures gave rise to the adrenal cortical neoplasms? (b) What structures were involved in the origin of those ovarian carcinomas that resembled the adrenal cortical tumors?

TABLE I

Spayed females					Castrated males			
		No. of mice with adrenal tumors					No. of mice with adrenal tumors	
Age, months	No. of mice	Unilateral	Bilateral	Lung metast.	Age, months	No. of mice	Unilateral	Bilateral
1	3	0	0		1	4	0	0
2	2	0	0		2	4	0	0
3	3	0	0		3	2	0	0
4	4	0	0		4	4	0	0
5	1	0	0		5	1	1	0
6	5	5	0		6	5	0	0
7	4	3	1		7	2	2	0
8	1	0	1		8	1	1	0
9	2	0	2		9	6	2	1
10	2	0	2		10	4	2	2
11	1	1	0		11	1	1	0
12	6	1	5	1	12	5	3	2
13	2	0	2		13	1	0	0
14	3	0	3	1	14	1	1	0
15	2	1	1		15	2	1	1
16	6	0	6	2	17	3	2	1
17	8	2	6		18	3	0	2
18	5	1	4	1	19	1	1	0
19	5	0	5	2	21	3	1	2
20	2	1	1		23	4	0	4
22	4	3	1	1	24	2	1	1
					25	1	1	0
					28	1	0	1
Total	71	18	40	8		61	20	17

TABLE II

Type of mice	No. of mice over 3 months of age	No. and % of adrenal tumors	Unilateral	Bilateral
Spayed females	63	58 91.90%	18 31.03%	40 68.96%
Castrated males	51	37 72.54%	20 54.05%	17 45.94%

The early changes and small tumors in the adrenals of the gonadectomized ce mice were found immediately beneath and in some cases between the fibers of the capsule. It is conceivable that the adrenals, which were retarded in development, contained some early components from the region of the genital ridge, and that these cells might have been kept undifferentiated and inhibited by sex hormones produced by functioning gonads. Perhaps they themselves were potentially capable of hormone production, and after the removal of the gonads attempted to compensate for them. Gonadectomy freed these undifferentiated cortical cells from inhibition and resulted in their uncontrolled proliferation. Likewise the ovaries may have contained similar cells from the genital ridge, which were inhibited when normal functioning ovaries were present. By this hypothesis a deficiency of sex hormone due to ovarian hypofunction may have resulted here, too, in an uncontrolled proliferation of the same type of cell.

If the above hypotheses are correct the proper sex hormone treatment might prevent the development of adrenal cortical tumors, or arrest them if they are already present. Further experiments should be carried out to test this assumption.

SUMMARY

Gonadectomy was performed at 2 days of age on 70 females and 61 males of the extreme dilution ce strain of mice. Adrenal cortical carcinomas arose in many of them.

The total percentage of adrenal tumors was 91.90 in the females and 72.54 in the males. There were 31.03 per cent of unilateral adrenal tumors in the females and 54.05 per cent in the males. Bilateral adrenal tumors occurred in 68.96 per cent of the females, and 45.94 per cent of the males. Only animals more than 3 months old are included in these figures.

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The Carcinogenicity of Certain Azo Dyes Related to *p*-Dimethylaminoazobenzene*

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(Received for publication October 10, 1944)

Information on the relation of molecular structure to carcinogenicity is not nearly so extensive for the azo dyes as for the polycyclic hydrocarbons (10). Most of the pertinent publications appeared in Japan shortly after the carcinogenicity of azo dyes was first recognized, but before experimental conditions were established for an adequate survival of animals ingesting these compounds. One of the most active investigators in this field has published in English only his conclusions, without sufficient data for an evaluation of the results (14-16). Virtually all the earlier assays were made with a basal diet consisting of rice and carrot. Although this particular dietary combination was useful in the original discovery of the carcinogens *o*-aminoazotoluene (35) and *p*-dimethylaminoazobenzene (14), and also in the discovery that the development of hepatic tumors varies with diet (27), it may be questioned whether its continued use has not actually hindered further progress. All too frequently the feeding of an azo dye with rice and carrot has resulted in so high a mortality before tumors developed that any conclusion based on the surviving animals was without quantitative significance. The deficiencies in this diet have been discussed elsewhere (23), and the progress made with other diets is a matter of record (4, 8, 21-24, 34). It is now possible to examine the carcinogenicity of a series of closely related derivatives of *p*-dimethylaminoazobenzene under uniform dietary conditions that will permit an adequate survival of the experimental animals. An extension of the older data appeared particularly desirable, because such information is necessary to evaluate an attempt made by Kensler, Dexter, and Rhoads (11) to explain how azo dyes produce tumors.

The essence of their idea is that it is not the azo dye that initiates the carcinogenic process, but an enzyme poison, the split product, formed in the breakdown of the "parent" azo compound in the body. The split product hypothesis is in harmony with the older idea that the tumor problem is essentially one of deranged enzyme systems, and to date it is the only mechanism capable of further experimental test that has been suggested for the action of the azo dyes. The evidence for the hypothesis is as follows: Kensler, Sugiura, and Rhoads (12) noted that the diphosphopyridine nucleotide content of rat livers diminished during the feeding of *p*-dimethylaminoazobenzene, and that the coenzyme was very low in the induced tumors. Kensler, Dexter, and Rhoads then tested the effect of the dye and of its possible metabolites on a yeast zymase system in which the coenzyme was the limiting factor. *p*-Dimethylaminoazobenzene itself, *p*-aminophenol, a known metabolite (31), and aniline, a hypothetical metabolite, did not inhibit this system. However, *p*-phenylenediamine, known to be formed in the body from the azo dye (31), and in particular its possible precursor, N,N-dimethyl-*p*-phenylenediamine, were found to be very toxic to this system at low concentrations. The inhibitory action in the zymase system was then determined for a series of ring- and N-methylated *p*-phenylenediamines that had been employed by Michaelis, Schubert, and Granick (18) in a study of the properties of the free radicals obtained in the one-step oxidation of these diamines. Kensler, Dexter, and Rhoads reached the conclusion that the toxicity of a given diamine paralleled the stability of its free radical and its ease of oxidation (by air and/or the crude zymase preparation).[1] Similar observations were made by Kensler, Young, and Rhoads (13) on a crude yeast carboxylase preparation. The next step (11) was to correlate the carcinogenic potency of a series of azo dyes with the toxicity of the diamine and the stability of the free radicals that were presumed to be formed from the dyes in the animal

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This investigation was aided by the Wisconsin Alumni Research Foundation and the Jonathan Bowman Cancer Fund. We are indebted to Miss Janet Giese for the care of some of the animals.

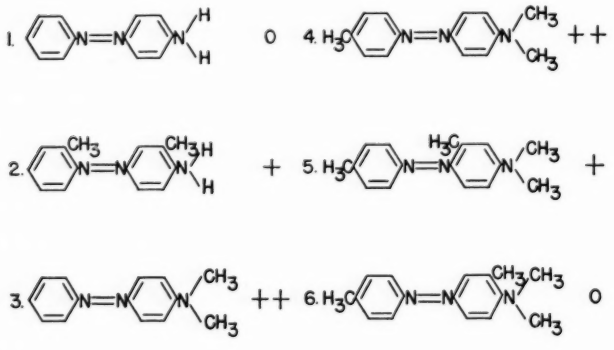
Presented in part at the meetings of the American Association for the Advancement of Science, Gibson Island, August 3, 1944.

** Finney-Howell Fellow, 1943-44.

[1] However, there appears to be some question whether the free radicals formed from the diamines are the enzyme poisons (11) or whether higher oxidation products such as *p*-quinones are the true inhibitors (17, 28).

body. The conclusion was drawn that if the *p*-diamine split product of a methyl derivative of *p*-dimethylaminoazobenzene inhibits the yeast zymase system employed by at least 65 per cent, and the stability of the free radical is more than 4 hours, the original azo compound can be expected to produce liver cancer in the rat.

The table of correlations published appears on the surface to bear out this statement. The stabilities of the free radicals of the diamines have presumably been determined accurately, and for all practical purposes the toxicities of the diamines toward enzyme systems may also be regarded as factually correct. But a critical examination of the evidence fails to substantiate the carcinogenic activities indicated for several of the azo dyes in the table. Of the 6 crucial compounds tabulated, only the first 3 have been



studied sufficiently to justify the assigned strengths. The latter 3 compounds were not fed at the usual 0.06 to 0.10 per cent levels but at 0.03 per cent for 45 days, then at 0.06 per cent for another 45 days, and finally at 0.2 per cent for the remainder of the experiments, which lasted 402 to 610 days (25). Thus compounds 4 to 6 were fed for long periods of time at levels 2 to 3 times as high as that employed for the first 3 dyes. Compound 4, *p'*-methyl-*p*-dimethylaminoazobenzene, was fed to 31 rats and 15 of these survived for 210 days, when the first tumor was discovered. The experiment was concluded in 402 days, at which time 12 tumors had appeared. This compound was assigned a potency of ++, the same as that given *p*-dimethylaminoazobenzene, compound 3. Actually compound 4 would appear to be considerably less active than *p*-dimethylaminoazobenzene, since 0.06 per cent of the latter dye gave 100 per cent tumors in 120 to 150 days (14, 23). Indeed, Nagao's compound 4 is probably no more active than compound 2, *o*-aminoazotoluene, and may even be less active when fed at comparable levels in the diet. Compound 5, *o,p'*-dimethyl-*p*-dimethylaminoazobenzene, an azo compound which on reduction could yield a *p*-diamine that is a less active enzyme poison than the diamine obtainable from compounds 3 or 4, was fed to 33 rats by Nagao (25). Fourteen survived 240 days on the diet and by 476 days only a few cysts were observed in the livers. At this time *one* liver was found to be tumorous, and the experiment was terminated at 610 days without any more tumors having been noted. Yet this compound was assigned a potency of +, a potency also assigned to the very definitely carcinogenic *o*-aminoazotoluene (10, 35). Compound 6, *m,p'*-dimethyl-*p*-dimethylaminoazobenzene, which on reduction could yield a diamine nontoxic in enzyme experiments (11, 13), was fed to 36 rats. Four survived 352 days, and none of these developed tumors by 610 days. This compound was assigned a potency of 0. However, while 3 references are given in Nagao's paper (25) for the preparation of the azo dyes employed, these references actually refer only to compounds 4 and 5. An examination of the chemical literature has failed to reveal any mention of compound 6. Since a methyl group *ortho* to the dimethylamino group, as in compound 6, strongly inhibits ordinary diazo coupling reactions (5, 7) except when the *p'* position is occupied by a strong negative group (1, 2, 18), it would appear that there is a reasonable doubt concerning the identity of the substance fed as compound 6.

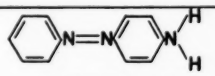
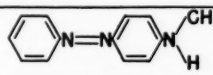
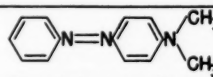
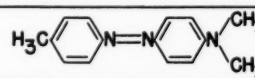
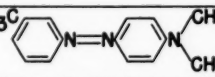
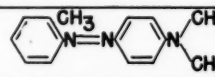
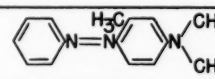
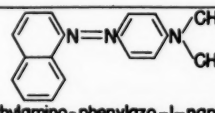
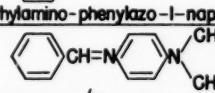
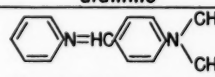
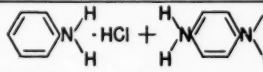
The data on compounds 4 to 6 are thus seen to be inadequate for even a rough comparison of carcinogenic potency with such established properties as the stability of free radicals or the ability of diamines to inhibit enzyme systems. The correlation originally published (11) therefore involves only 2 azo dyes closely related to *p*-dimethylaminoazobenzene whose potencies are known with any degree of certainty: *o*-aminoazotoluene, and *p*-aminoazobenzene. The need for more extensive data thus becomes obvious. Toward this end 11 compounds allied to *p*-dimethylaminoazobenzene were prepared and tested for their carcinogenic activity in rats fed a suitable basal ration. Particular emphasis was placed upon monomethyl derivatives of the dye that could theoretically yield the same diamine as *p*-dimethylaminoazobenzene, but differed in the configuration of the other half of the molecule.

Preparation of Compounds [2]

The compounds tested are listed in Table I. The *p*-dimethylaminoazobenzene and the *p*-dimethylaminophenylazo-1-naphthalene were Eastman products. The

[2] Substituents of *p*-dimethylaminoazobenzene have been designated as *o*, and *m* on the ring bearing the $-N(CH_3)_2$ group and as *o'*, *m'*, and *p'* on the other ring, starting in each case from the azo linkage. The corresponding *p*-diamines have been named in a similar fashion.

TABLE I: THE CARCINOGENICITY OF CERTAIN AZO DYES RELATED TO *p*-DIMETHYLAMINOAZOBENZENE

Diet no.	Compound fed	Per cent in diet	Time compound was fed mos.	Average weight increment during feeding of compound gm./rat	Incidence of liver tumors*				
					3 mos.	4 mos.	6 mos.	8 mos.	10 mos.
1	 *p*-Aminoazobenzene	0.053	8	+81				0/9	0/9
2	 *p*-Monomethylaminoazobenzene	0.056	4	+17	not palpable	7/8	8/8		
3	 *p*-Dimethylaminoazobenzene	0.060	4	+5	"	6/8	8/8		
4	 *p'*-Methyl-*p*-dimethylaminoazobenzene	0.064	8	+56				0/10	1/10
5	 *m'*-Methyl-*p*-dimethylaminoazobenzene	0.064	3	−24	5/8	7/8			
6	 *o'*-Methyl-*p*-dimethylaminoazobenzene	0.064	6	+13			2/9	4/9	
7	 *o*-Methyl-*p*-dimethylaminoazobenzene	0.064	8	−10				0/8	0/8
8	 *p*-Dimethylamino-phenylazo-1-naphthalene	0.073	8	+62				0/8	0/8
9	 *N,N*-Dimethyl-*N'*-benzal-*p*-phenylene-diamine	0.060	8	+74				0/10	0/10
10	 *p*-Dimethylaminobenzal-aniline	0.060	8	+104				0/9	0/9
11	 Aniline *N,N*-Dimethyl-*p*-phenylene-diamine	0.035 and 0 056 resp.	8	+92				0/9	0/9
12	*p*-Dimethylaminoazobenzene	0.006	8	+68				0/8	0/8
13	Azo dye as in diet 12 plus split-products as in diet 11	as in diets 11 and 12	8	+74				0/10	0/10

* Incidence = Number with tumors over number surviving when laparotomies were performed.

Schiff bases, N,N-dimethyl-N′-benzal-*p*-phenylenediamine and *p*-dimethylaminobenzal aniline were prepared as directed by Calm (3), and Sachs and Lewin (29) respectively. The split product of *p*-dimethylaminoazobenzene, N,N-dimethyl-*p*-phenylenediamine, was prepared by the reduction of *p*-nitrosodimethylaniline (6). This diamine and aniline were fed as the hydrochlorides. *p*-Aminoazobenzene and *p*-monomethylaminoazobenzene were prepared as described previously (19). The remaining compounds, all monomethyl derivatives of *p*-dimethylaminoazobenzene, were prepared by diazotizing 1 mole of the appropriate amine with 1 mole of sodium nitrite in the presence of 2½ moles of hydrochloric acid at 0° C. and adding the diazo solution to a 70 per cent ethanol solution of 1 mole of the desired tertiary amine in the presence of 2 moles of sodium acetate. The precipitated azo dye was then recrystallized from ethanol-water solution. The melting points of the azo dyes and the Schiff bases agreed with those reported in the literature.

All the 5 possible ring-monomethyl derivatives of *p*-dimethylaminoazobenzene were prepared and tested, with the exception of *m*-methyl-*p*-dimethylaminoazobenzene. This compound is mentioned only once in the literature, by Hantzsch (9), who stated merely that though it had not been described previously he had prepared it ". . . *auf die übliche Weise* . . ." (in the usual way). It is doubtful, however, that the usual direct coupling procedures were meant, since *o*-methyl-N,N-dimethylaniline does not couple appreciably with benzene diazonium salts (5, 7); coupling with this amine occurs only when the diazonium compound is activated by a strong negative group, *e.g.*, $—NO_2$, $—SO_3H$, in the *para-* position (1, 2, 18). Alternative procedures for the preparation of *m*-methyl-*p*-dimethylaminoazobenzene have been attempted in this laboratory without success.

METHODS

Each compound was fed to 10 young adult albino rats 150 to 200 gm. in weight. The rats were kept in groups of 5 in screen-bottomed cages, and food and water were given *ad libitum*. The basal diet was a semisynthetic one of the following composition: crude casein, 120 gm.; salts, 40 gm.; corn oil, 50 gm.; rice bran concentrate, 20 gm.; glucose, 770 gm.; and riboflavin, 0.5 mgm. per kilogram. Each rat received 1 drop of halibut liver oil monthly. This diet produces nearly 100 per cent liver tumors when 0.06 per cent of *p*-dimethylaminoazobenzene is fed for 4 months (23). In the present experiments each compound was fed at molar levels equivalent to the usual carcinogenic level of 0.06 per cent of *p*-dimethylaminoazobenzene. The azo dyes and the Schiff bases were incorporated in the diet by dissolving them with heat in the corn oil. The hydrochlorides of the split products of *p*-dimethylaminoazobenzene were mixed with the dry constituents of the diet. The rats were fed these diets until tumors developed or until 8 months had elapsed. Laparotomies were performed when palpable tumors formed or at 8 months. The rats were then fed the basal diet without the compounds for another 2 months, to allow any latent tumors to develop (23), and a final examination of the livers was made at the end of this period. No attempt was made to ascertain the ultimate carcinogenicity of any of the compounds that proved weakly active or inactive in this study. The arbitrary feeding period of 8 months is twice as long as the period of 4 months generally used with the control compound, *p*-dimethylaminoazobenzene.

RESULTS

The incidence of liver tumors in the rats fed these compounds is presented in Table I. Most of the rats on the various diets gained in weight, their general physical condition remained good, and the survivals ranged from 80 to 100 per cent. Under the conditions of the experiment, 4 compounds other than *p*-dimethylaminoazobenzene were carcinogenic. Together with the high survival in each group this indicates that the inactivity of the remaining compounds is also real under these conditions. The incidence of tumors in the rats fed *p*-dimethylaminoazobenzene (Diet 3) was 88 per cent at 4 months and 100 per cent at 6 months. This incidence is normal, and has been observed many times when the compound was fed in the basal diet employed (23, 24).

The completely demethylated derivative, *p*-aminoazobenzene, was inactive by 10 months (Diet 1). This agrees with the observation of Sasaki and Yoshida (30) that rats fed 0.1 per cent of the dye for 14 months did not develop tumors. The partially demethylated derivative, *p*-monomethylaminoazobenzene, was found to be practically as active as the dimethyl compound (Diets 2 and 3), and its carcinogenic potency has been confirmed by subsequent experiments. The similar carcinogenic potencies of these 2 compounds have counterparts in the known metabolism of the 2 dyes; essentially the same concentrations of these 2 dyes and of *p*-aminoazobenzene occurred in the livers of rats fed either the dimethyl or the monomethyl azo compound (20).

The ring-monomethyl derivatives of *p*-dimethylaminoazobenzene tested ranged in activity from very weak, or moderate, to very high activity. One of the 4, *o*-methyl-*p*-dimethylaminoazobenzene, did not produce any tumors after 10 months (Diet 7). Nagao (26) fed this compound as the hydrochloride at the

very high levels of 0.4 to 2.5 per cent and obtained tumors after nearly 12 months.[3] *p'*-Methyl-*p*-dimethylaminoazobenzene (Diet 4) produced only 1 tumor in 10 rats at 10 months. Nagao (25) fed 0.2 per cent of this dye and obtained liver tumors in 12 of 15 rats by 13 months. *o'*Methyl-*p*-dimethylaminoazobenzene, a compound not tested previously, produced liver tumors in 4 of 9 rats by 8 months (Diet 6).

The activity of the last ring-methyl compound, *m'*-methyl-*p*-dimethylaminoazobenzene, has not been recorded before. Surprisingly this compound proved to be more active (Diet 5) than the parent compound, *p*-dimethylaminoazobenzene. Large tumors were palpated and confirmed by laparotomy in 5 of 8 rats at 3 months, at which time no tumors could be palpated in the rats fed any of the other dyes. By 4 months 7 of the 8 rats had tumors, a negative animal having died in the interim. This dye thus had produced tumors approximately 1 month sooner than *p*-dimethylaminoazobenzene. The high carcinogenicity of the *m'*-methyl derivative, which is also more toxic than the parent dye, has been confirmed in subsequent experiments in this laboratory. The simultaneous substitution of the *o'*- and *m'*- positions by a benzo group, as in *p*-dimethylamino-phenylazo-1-naphthalene, produced an inactive compound (Diet 8). In general, the severity of the toxicity and cirrhosis observed with these dyes paralleled their carcinogenic potencies. Gross cirrhosis was noted only on Diets 2, 3, 5, and 6.

The two Schiff bases (Diets 9 and 10) tested the possibility that the substitution of a $-CH=$ group for the $-N=$ atom in the azo linkage might produce noticeably different results, depending upon the position of the $-CH=$ group; for on cleavage only the first Schiff base (Diet 9) could yield the hypothetical split-product of *p*-dimethylaminoazobenzene. However, both compounds failed to produce liver tumors. But it is doubtful whether more than small amounts of either reached the liver, since Schiff bases are hydrolyzed by weak acids and this may have occurred in the stomachs of the rats. When the first Schiff base (Diet 9) was fed to rats, the diamine portion was found to be excreted in the urine (see below). A benzaldehyde-like odor was evident in diets containing the second Schiff base (Diet 10).

No tumors resulted by 10 months when the split-products of *p*-dimethylaminoazobenzene, *viz.*, aniline, and N,N-dimethyl-*p*-phenylenediamine (Diet 11), were fed as the hydrochlorides at levels equivalent to 0.06 per cent of the parent azo dye. These amines have been fed previously to rats for long periods of time without the formation of tumors (14, 33). As discussed below, part of the diamine fed was excreted unchanged in the urine. Diets 12 and 13 represent an attempt to discover whether the split products of *p*-dimethylaminoazobenzene possess cocarcinogenic activity. When 0.006 per cent of the azo dye, one-tenth of the usual carcinogenic level, was fed, no tumors resulted after 10 months (Diet 12). Maruya (10) produced 2 tumors in rats fed this level of the dye for 12 months. When 0.006 per cent of *p*-dimethylaminoazobenzene and levels of the split products equivalent to the carcinogenic level of this dye were fed together (Diet 13), the tumor incidence still remained at zero. No toxic symptoms were observed in rats fed the split products, and these rats were among the healthiest throughout the experiment.

An attempt was also made to test the carcinogenicity of *p,p'*-tetramethyldiaminoazobenzene, a compound that on reduction could yield 2 molecules of the hypothetical split product of *p*-dimethylaminoazobenzene. However, this compound, prepared according to Vorländer and Wolferts (32), was so insoluble that difficulty was experienced in dissolving it with heat in corn oil. Furthermore, as evidenced by its red color in strong HCl, considerable amounts of the dye were excreted in the feces. Since the compound thus did not appear to be well absorbed and in addition was difficult to prepare in quantity, feeding was discontinued after only a few weeks.

The "pine chip reaction."—The pine shavings beneath the cages of rats fed N,N-dimethyl-*p*-phenylenediamine as the hydrochloride or as a Schiff base (Diets 9, 11 and 13) were found to be stained purplish-red by the urine shortly after the compounds were fed. When pure water solutions of the diamine hydrochloride were applied to the pine chips, the same color developed. Aqueous solutions of the hydrochlorides of the allied diamines, *p*-phenylenediamine and N-monomethyl-*p*-phenylenediamine produced orange and reddish stains respectively when applied to pine chips. These compounds were also fed to rats and in every case the shade of color observed when the urine came into contact with the pine shavings was the same as that of the compound fed. Preliminary data indicated that the amount excreted was only a small percentage of that ingested; and no similar excretion of chromogen was noted in rats fed the parent azo dye, *p*-dimethylaminoazobenzene. The ingestion of the dye did not interfere with the excretion of the diamine, since rats fed a diet containing 0.06 per cent of the dye plus equivalent levels of the split products excreted urine that gave a typical

[3] No data are given in Nagao's paper (26) on the percentage of survival or on the number of tumors occurring in rats fed these huge doses of dye. The hydrochloride was used because, as Nagao states, the free base underwent chemical changes; in our experiments the pure free base was stable and the dye was fed in this form. We are indebted to Mr. K. Higuchi for the translation of this reference.

reaction with pine chips. Incidentally, the diamines were found to give similar colors with brown paper towelling, and to some extent with alcoholic solutions of vanillin. Filter paper, however, failed to give any color with the diamines. The colors formed in this reaction closely resembled those of the respective free radicals. However, the pigments produced in the wood were not soluble in water.

Apparently, therefore, the rats fed N,N-dimethyl-*p*-phenylenediamine were excreting part of the original diamine as such. This observation is of interest in connection with the failure of tumors to develop when the amines corresponding to the split products of *p*-dimethylaminoazobenzene are fed (14, 33; and Table I, diets 11 and 13), for if N,N-dimethyl-*p*-phenylenediamine were the true carcinogen tumors should develop after the substances reached the susceptible liver cells. It might be postulated that the theoretically active diamine fails to reach the liver when it is fed as the hydrochloride, and that it reaches the proper liver cells only when the parent azo dye is fed. However, the finding of N,N-dimethyl-*p*-phenylenediamine in the urine of rats fed this substance is strong presumptive evidence that the diamine was widely distributed in the body. Indeed, since urine from rats fed *p*-dimethylaminoazobenzene failed to give a pine chip reaction, it would appear that more diamine is present in the tissues after the feeding of the diamine itself than after the feeding of the azo dye.

Demethylation of p-dimethylaminoazo dyes in vivo.—Preliminary analyses were made to determine whether the *p*-dimethylaminoazo dyes other than *p*-dimethylaminoazobenzene listed in Table I also undergo demethylation *in vivo* prior to cleavage at the azo linkage. The methods employed have been described previously (19, 20). Three separate azo dyes were found to be present in the livers of rats fed each dye, and these corresponded closely in their adsorption characteristics to the derivatives obtained from *p*-dimethylaminoazobenzene (20). The blood in each case contained only one compound which, from its chromatographic behavior, appeared to be the completely demethylated derivative. When *o*-aminoazotoluene was fed, only one compound, presumably the original dye, could be found in the liver and blood. Apparently, therefore, demethylation prior to fission of the azo group is a characteristic common to the metabolism of many *p*-dimethylaminoazo dyes.

DISCUSSION

The carcinogenic potencies found for the *o'*-, *m'*-, and *p'*- methyl derivatives of *p*-dimethylaminoazobenzene support the split-product theory of carcinogenesis (11) only in so far as each compound in the series caused some tumors to develop. But the wide quantitative variations in carcinogenicity observed among the members of this group, each of which could theoretically yield the same diamine on cleavage, indicate that the other non-diamine half of the parent molecule also exerts an important effect on the carcinogenicity of an azo dye. The *m'*- methyl compound was definitely more active than *p*-dimethylaminoazobenzene, the *o'*- methyl compound was roughly half as active, while the *p'*- methyl derivative was only very weakly carcinogenic.

Other examples of a lack of correlation between carcinogenicity and the toxicity of the corresponding diamine are as follows: *o*-methyl-*p*-dimethylaminoazobenzene is only weakly carcinogenic (26; and Table I, Diet 7), much less so than might be expected from the toxicity and stability of the free radical of the corresponding diamine, *o*-methyl-N,N-dimethyl-*p*-phenylenediamine [2] (11, 18). Furthermore, the carcinogenicity of *p*-monomethylaminoazobenzene is equal to that of *p*-dimethylaminoazobenzene, although the toxicity and free radical stability of the corresponding diamine (11) would lead one to predict a lower degree of carcinogenicity for the monomethyl compound, if the stability of the free radical were the determining factor. It would have been desirable to test the carcinogenicity of *m*-methyl-*p*-dimethylaminoazobenzene for on reduction this substance could yield a nontoxic diamine, *m*-methyl-N,N-dimethyl-*p*-phenylenediamine (11), and hence, according to the criteria of Kensler and his group, the parent azo dye should be noncarcinogenic.

The discrepancies enumerated are probably best judged in the light of analytical results on the metabolic fate of azo dyes in the body of the rat. Both *p*-dimethyl- and *p*-monomethylaminoazobenzene are demethylated in the animal prior to cleavage at the azo linkage (20). The monomethyl derivative can also be methylated to yield the dimethyl compound, and the quantitative distribution of azo dyes in the tissues, and especially in the liver, is essentially the same whether *p*-monomethyl- or *p*-dimethylaminoazobenzene is given (20). Since the rate of tumor formation is also essentially the same when either dye is fed, it would appear that the concentration of azo dye in the liver, rather than the stability of a hypothetical cleavage product, is the determining factor in carcinogenesis with these azo dyes. Indeed, the concentration of the completely demethylated product, *p*-aminoazobenzene, in the tissues is essentially the same whether this dye or either of its two carcinogenic N-methyl derivatives is fed, suggesting that most of the carcinogen given may be demethylated prior to cleavage in the animal body, thus precluding the formation of appreciable amounts of the hypothetical N,N-dimethyl-*p*-phenylenediamine.

Hence neither of the two new lines of evidence, the analytical data or the carcinogenicity of related azo dyes, supports the split-product theory of carcinogenesis. It must also be stated, however, that these results do not completely rule out the possibility of N,N-dimethyl-*p*-phenylenediamine functioning as originally postulated (11). For even if 99 per cent of *p*-dimethylaminoazobenzene should be demethylated prior to cleavage, it would still be possible that the remaining fraction could yield the cleavage product postulated (although proof to that effect ought to be furnished). The demonstration of N,N-dimethyl-*p*-phenylenediamine in the urine of rats fed this substance suggests its presence in the body fluids, but does not prove it to be within the liver cells. The lack of correlation between the postulated carcinogenicity of the various methyl derivatives of *p*-dimethylaminoazobenzene and that observed could have been due to secondary complications, such as, *e.g.*, unequal efficiencies of transportation from the gut to the liver depending upon other groupings in the molecule. Indeed it might even be argued that if carcinogens can undergo metabolic reactions unrelated to the carcinogenic process, the number of tumors obtained is not necessarily an index of the carcinogenicity of the molecule tested. This would, however, add still another difficulty to attempts at establishing a table of correlations.

The present status of the split-product theory, therefore, appears to be that there is enough circumstantial evidence against the theory to raise the question of its validity. But whether or not the original theory ultimately prevails is probably of secondary consequence; it has already furnished a stimulus for much experimental work, and at least one concrete result is the discovery of the most carcinogenic azo dye known, *m'*-methyl-*p*-dimethylaminoazobenzene. We are unaware of any evidence against the view that the intact azo molecule is the true carcinogen. In contrast to this, however, an extreme opposite view should also be stated: Kuhn and Beinert (17) believe that the carcinogenicity of these azo dyes depends on the ultimate oxidation product, benzoquinone, or its methyl derivative, rather than upon the diamine or its free radical.

SUMMARY

1. Eleven compounds closely related to *p*-dimethylaminoazobenzene were fed to rats at levels equivalent to 0.06 per cent of this dye in an adequate semisynthetic diet for periods up to 240 days, and their potencies as liver carcinogens noted up to 300 days.

2. *m'*-Methyl-*p*-dimethylaminoazobenzene proved to be more active than *p*-dimethylaminoazobenzene. *p*-Monomethylaminoazobenzene was practically as active as the parent compound. *o'*-Methyl-*p*-dimethylaminoazobenzene and *p'*-methyl-*p*-dimethylaminoazobenzene in that order were less active than *p*-dimethylaminoazobenzene.

3. The remaining 7 compounds, *p*-aminoazobenzene, *o*-methyl-*p*-dimethylaminoazobenzene, *p*-dimethylamino-phenylazo-1-naphthalene, N,N-dimethyl-N'-benzal-*p*-phenylenediamine, *p*-dimethylaminobenzal aniline, N,N-dimethyl-*p*-phenylenediamine, and aniline were inactive under the conditions employed.

4. Aqueous solutions of the hydrochlorides of *p*-phenylenediamine and its N-monomethyl and N,N-dimethyl derivatives were found to give characteristic stains on pine chips. By this test rats fed the split product of *p*-dimethylaminoazobenzene, N,N-dimethyl-*p*-phenylenediamine, were found to excrete some of this diamine as such in the urine. No similar excretion of chromogen was noted in rats fed the azo dye.

5. Each of the *p*-dimethylaminoazo dyes tested underwent a stepwise demethylation *in vivo* prior to cleavage at the azo linkage.

Our data are not in accord with the theory of Kensler, Dexter, and Rhoads on the means by which azo dyes produce liver cancer.

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Observations on Rats Fed with Compounds Related to Dimethylaminoazobenzene

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(Received for publication, November 6, 1944)

Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat and to a lesser extent in other species (1, 3-5). N,N-dimethyl-*p*-aminoazobenzene has been found to be the most potent carcinogen of this type so far tested (3, 6).

It is our purpose in this paper to report observations concerning relative carcinogenic activity in the rat of several compounds of this series that have not been previously tested.

EXPERIMENTAL

A 3 per cent solution of N,N-dimethyl-*p*-aminoazobenzene or molar equivalent amounts of other compounds was prepared, and 20 cc. were evenly mixed with 1,000 gm. of coarsely ground, unpolished rice; *i. e.*, 0.6 gm. of the compound per kg. The rats were permitted to eat as much of the mixture as they desired; all received a small amount of fresh carrots daily and unlimited quantities of water were permitted. Feeding was continued until the animals either succumbed or were sacrificed. The rats used in this study were of the Sherman stock, with an initial body weight of about 150 gm.

The results obtained are summarized in Table I. In all cases the gross diagnosis was confirmed by microscopic examination.

As may be seen, the daily ingestion of large amounts of N,N-diethyl-*p*-aminoazobenzene, N,N-dipropyl-*p*-aminoazobenzene, N,N-dibutyl-*p*-aminoazobenzene, and N,N-diamyl-*p*-aminoazobenzene, the higher homologues of N,N-dimethyl-*p*-aminoazobenzene, failed to produce liver cirrhosis or tumors in rats during a feeding period lasting 78 to 511 days. On the other hand, the animals fed N,N-dimethyl-*p*-aminoazobenzene developed typical liver cancer in all cases in comparatively shorter periods of time.

It is interesting to note that approximately the same degree of carcinogenic activity is attained by the N-methyl compound as by the N-dimethyl derivative. Cholangiomas (bile duct carcinoma) or hepatomas developed in all 10 rats fed N-methyl-*p*-aminoazobenzene for 108 to 228 days (Fig. 1). Two of the rats also had metastases to the mesentery and omentum. It was found, however, that when the methyl group is attached to the second phenyl ring (*p*) instead of to the amino group, the carcinogenicity is greatly reduced. Of 12 rats that received 4-methyl-4′-aminoazobenzene orally only 1, which died 427 days after the beginning of the experiment, showed a liver with neoplastic growth. The organ was covered with large cancer nodules, and metastases to the mesentery, omentum, and diaphragm were found; 11 rats, or 92 per cent, had smooth, practically normal livers.

Although 3,2′-dimethyl-4-aminoazobenzene (*o*-aminoazotoluene) is highly carcinogenic, 2′,3′,4′,5′,6′-pentamethyl-4-aminoazobenzene is noncarcinogenic.

During the course of this study the effect upon the rat liver of feeding a combination of aniline and N,N-dimethyl-*p*-phenylenediamine, split products of N,N-dimethyl-*p*-aminoazobenzene, was investigated. Molecular equivalent amounts of these substances were well mixed with unpolished rice and fed in the usual manner. The results of this experiment showed that the daily ingestion of large amounts of these substances failed to produce liver cirrhosis or tumors in rats during experimental periods of 147 to 515 days.

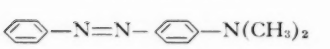
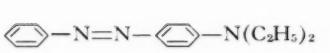
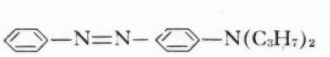
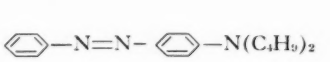
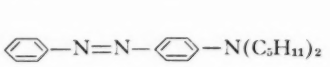
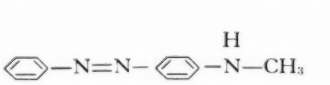
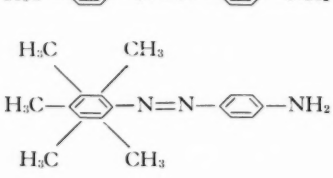
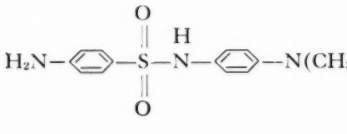
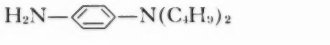
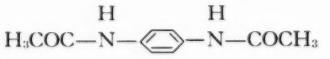
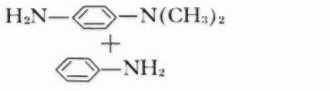
Diacetyl-*p*-phenylenediamine, one of the metabolites of N,N-dimethyl-*p*-aminoazobenzene, was similarly tested for its carcinogenicity. None of the 10 rats fed the acetylated paraphenylenediamine showed cirrhosis or neoplastic changes in the liver. Two of these animals lived more than 240 days.

The liver of a rat fed N,N-diethyl-*p*-aminoazobenzene showed a few flecks. Microscopic sections of the liver showed neither cirrhosis nor tumor.

While the livers of animals fed N,N-diethyl-*p*-aminoazobenzene and N,N-dipropyl-*p*-aminoazobenzene showed no great changes in color, size, or shape many of the livers of animals fed N,N-dibutyl-*p*-aminoazobenzene and N,N-diamyl-*p*-aminoazobenzene were pale and yellowish in color. The livers had smooth surfaces and histological examination showed no evidence of tumors, bile duct changes, or abnormal regeneration of the ducts and liver cells, or any abnormal nuclear alterations.

The only visceral changes seen were those arising from bronchopneumonia. No tumor was found in the visceral organs other than the liver or elsewhere, except that the stomach of one rat fed N,N-dibutyl-*p*-phenylenediamine for 546 days showed papillomas in the forestomach and diffuse hemorrhage in the glandular region of the stomach.

Table I: Incidence of Hepatic Tumors in Rats Fed Various Azo and Related Compounds

Chemical compound	Structural formula	No. of animals	Amount ingested daily, mgm.	No. of days fed	Liver findings *					Incidence of liver cancer, %
					−	±	+	++	+++	
N,N-Dimethyl-*p*-aminoazobenzene ‡		34	4.8	100-280	0	0	3	20	11	100
N,N-Diethyl-*p*-aminoazobenzene		10	4.8	88-511	10	0	0	0	0	0
N,N-Dipropyl-*p*-aminoazobenzene		10	4.8	78-207	10	0	0	0	0	0
N,N-Dibutyl-*p*-aminoazobenzene		10	4.8	97-496	10	0	0	0	0	0
N,N-Diamyl-*p*-aminoazobenzene		10	4.8	134-468	10	0	0	0	0	0
N-Methyl-*p*-aminoazobenzene		10	4.8	108-228	0	0	2	6	2	100
4′-Methyl-4-aminoazobenzene		12	4.8	73-768	11	0	0	0	1	8
2′,3′,4′,5′,6′-Pentamethyl-4-aminoazobenzene		12	7.9	106-286	12	0	0	0	0	0
p-Sulfanilylamido-dimethylaniline		5	6.5	146-493	5	0	0	0	0	0
N,N-Dibutyl-*p*-phenylenediamine		5	6.5	143-546	5	0	0	0	0	0
Diacetyl-*p*-phenylenediamine		10	5.0	100-240	10	0	0	0	0	0
N,N-Dimethyl-*p*-phenylenediamine plus aniline		15	4.4 2.8	147-515	15	0	0	0	0	0

* − indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma or both; ++ indicates extensive liver cancer without metastasis; +++ indicates extensive liver cancer with metastasis.

‡ N,N-Dimethyl-*p*-aminoazobenzene, No. 338, Eastman Kodak Company.

All the alkyl radicals were of the normal series.

DISCUSSION

Of the new compounds tested for carcinogenic potency in the rat by oral administration, only one, N-methyl-*p*-aminoazobenzene, was found to be highly carcinogenic. An isomer of this compound, 4′-methyl-4-aminoazobenzene, was only slightly carcinogenic (only 1 rat out of 12 showed cirrhotic and neoplastic changes). The carcinogenic potency of these 3 compounds correlates relatively well with the *in vitro* "toxicity" of the diamine split products (2).

All the higher alkyl homologues of N,N-dimethyl-*p*-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the rat when fed in equimolar amounts.

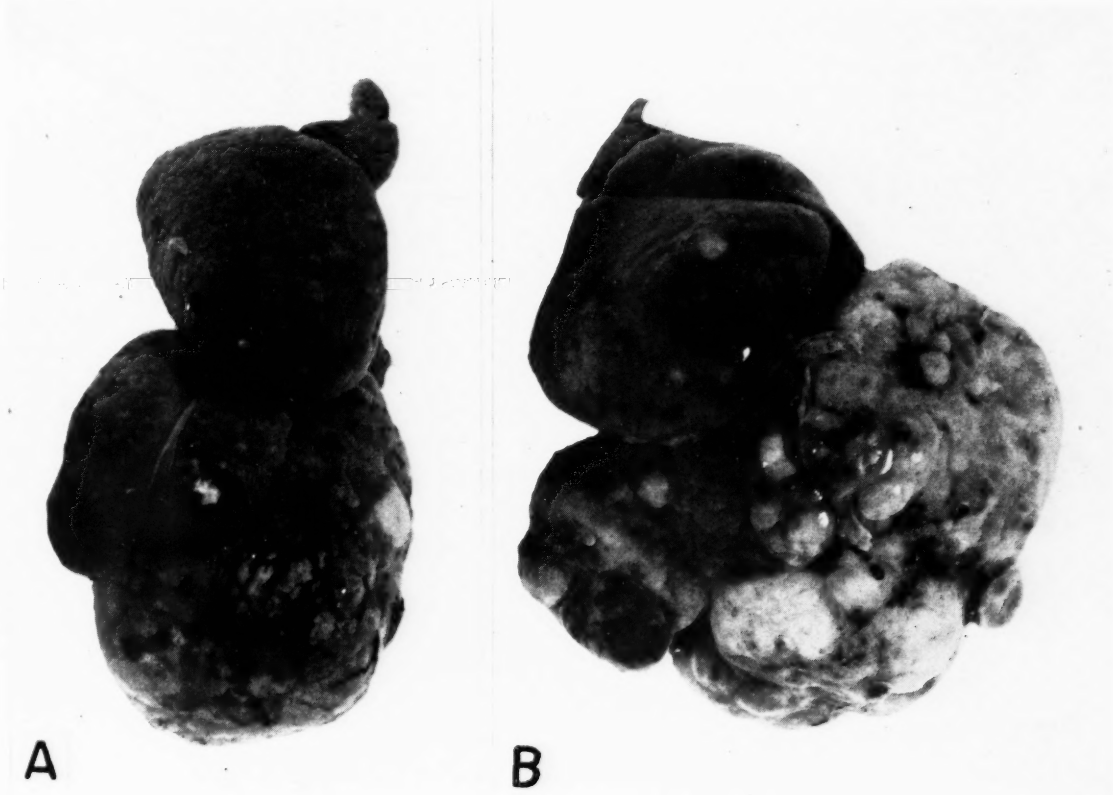


FIG. 1.—Gross appearance of livers (A and B) of rats fed N-methyl-*p*-aminoazobenzene-rice diet for 150 and 228 days respectively, showing cholangioma and hepatoma nodules.

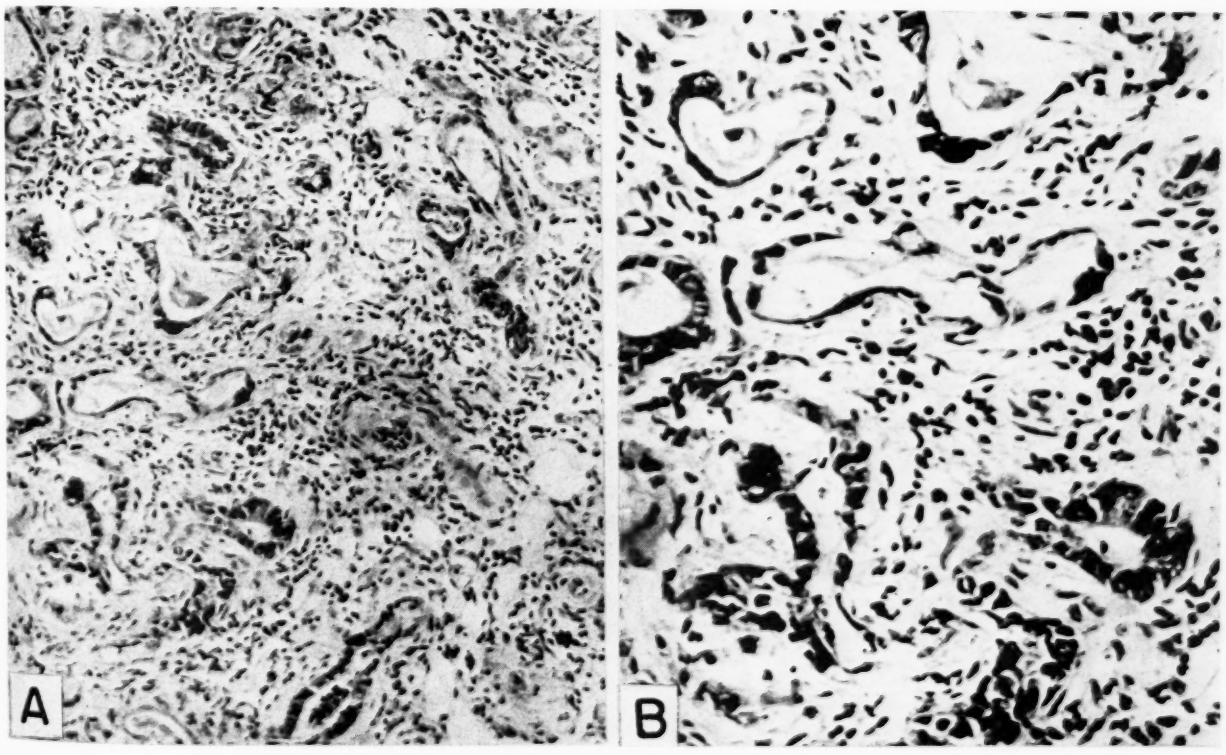


FIG. 2.—A. Section of liver showing cholangiomatous type of tumor. Hematoxylin and eosin. Mag. × 90. (For gross liver see Fig. 1-A.) B. Same section at higher magnification. Mag. × 210.

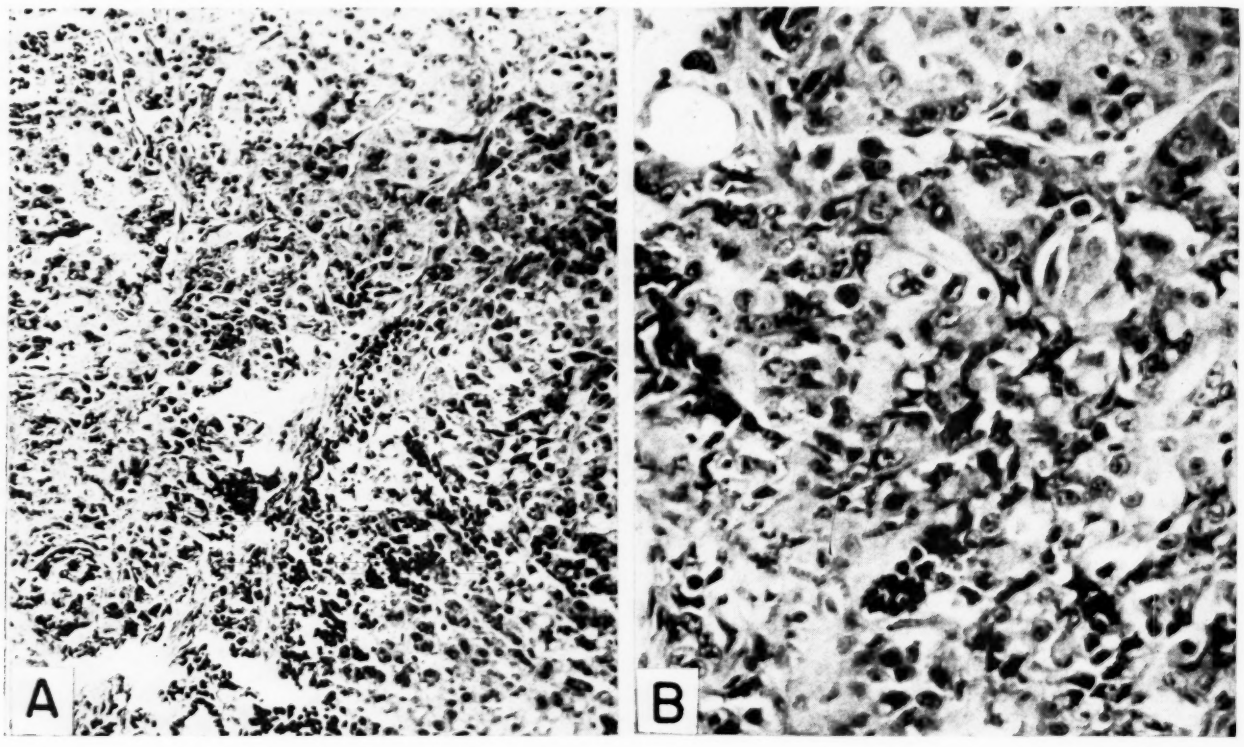


FIG. 3.—A. Lower power magnification of trabecular hepatoma in rat that received N-methyl-*p*-aminoazobenzene-rice diet for 228 days. Hematoxylin and eosin. Mag. × 90. (For gross liver see Fig. 1-B.) B. Same section at higher magnification. Mag. × 210.

Thus whereas methylation of the amino nitrogen of *p*-aminoazobenzene renders the molecule highly carcinogenic, alkylation with ethyl, propyl, butyl, and amyl groups does not.

To date no attempts to produce liver cancer in the rat by the administration of split products and related compounds of N,N-dimethyl-*p*-aminoazobenzene have been successful.

SUMMARY

1. All rats fed 4.8 mgm. of N,N-dimethyl-*p*-aminoazobenzene daily for a period of 150 days developed liver cancer.

2. Daily ingestion of 4.8 mgm. of N,N-diethyl-*p*-aminoazobenzene, N,N-dipropyl-*p*-aminoazobenzene, N,N-dibutyl-*p*-aminoazobenzene, or N,N-diamyl-*p*-aminoazobenzene failed to produce liver cancer, an indication of the importance of the methyl radical for carcinogenesis.

3. Although N-methyl-*p*-aminoazobenzene is highly carcinogenic its isomer, 4′-methyl-4-aminoazobenzene, is only feebly so.

4. Daily ingestion of 2′,3′,4′,5′,6′-pentamethyl-4-aminoazobenzene, *p*-sulfanilylamidodimethylaniline, N,N-dibutyl-*p*-phenylenediamine, diacetyl-*p*-phenylenediamine, and combined feeding with N,N-dimethyl-*p*-phenylenediamine and aniline, failed to produce liver cirrhosis or tumors in rats.

The authors wish to thank Professor L. F. Fieser and Dr. E. Berliner, of Harvard University, for the samples of the N-methyl-*p*-aminoazobenzene, 4′-methyl-4-aminoazobenzene, and 2′,3′,4′,5′,6′-pentamethyl-*p*-aminoazobenzene used in this investigation. We wish also to thank Dr. M. L. Crossley, of Calco Chemical Division, American Cyanamid Company, for N,N-diethyl-*p*-aminoazobenzene, N,N-dipropyl-*p*-aminoazobenzene, N,N-dibutyl-*p*-aminoazobenzene, N,N-diamyl-*p*-aminoazobenzene, N,N-dibutyl-*p*-phenylenediamine, and *p*-sulfanilylamidodimethylaniline; Dr. Leonor Michaelis, of The Rockefeller Institute for Medical Research, for N,N-dimethyl-*p*-phenylenediamine; and Dr. E. S. Stevenson, of Memorial Hospital, for diacetyl-*p*-phenylenediamine.

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The Effect of Insoluble Radiophosphorus (Chromium Phosphate) When Applied Interstitially in the Treatment of Adenocarcinoma of the Mamma in Mice*

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(Received for publication October 10, 1944)

Radioactive isotopes are metabolized by the body in exactly the same way as are the normally occurring stable isotopes of the same element. By the use of special technics involving sensitive methods of detection, much has been learned of the fate of substances "tagged" by means of radioactive isotopes.

Tracer studies with radiophosphorus have been particularly revealing because they have made possible the therapeutic action of this material in many blood dyscrasias. When radiophosphorus is injected in the form of the disodium salt, the phosphate radicle can be found throughout the body. It not only persists in the plasma and tissue fluids as phosphate, but is deposited in the bone as tricalcium phosphate. It is also synthesized into more complex phosphorus compounds such as hexosephosphates and phosphonucleotides. From these studies it is easy to understand why the bone takes up the greatest amount of injected radiophosphorus. There are also large amounts of phosphorus taken up by the soft tissues, especially those with high phosphonucleotide content.

In view of the fact that the cells of the hematopoietic system possess a relatively high phosphorus metabolism they are extremely active in their reception of the radioactive isotope. For the most part these cells are also extremely radiosensitive. As a result of this, when large amounts of radioactive disodium phosphate are administered its concentration in the bone and in the cells of the bone marrow may lead to considerable tissue destruction.

The clinical utilization of this principle in the treatment of leukemias, polycythemia vera, and lymphosarcoma has been encouraging in its results. The recent publication of Low-Beer, Lawrence, and Stone (4) will serve as an excellent reference for the subject.

Because of the rapidity of cell division in other types of malignant neoplastic disease, it was hoped that such a method of therapy would also be of great value in the management of various cancers and their metastases. Unfortunately the results have been discouraging. Because of the sensitivity of the bone marrow to radiation and the high concentration of radiophosphorus that occurs both in the marrow and in the bone, signs and symptoms of blood cell damage develop before there is much evidence of destruction of the tumor. It has been pointed out that the response of a tumor to radiophosphorus depends not only on the amount of radioactivity deposited in the tumor but also upon the radiosensitivity of its cells. Thus another type of tumor may presumably take up the same amount of radiophosphorus from the blood stream as, for example, a nodule of lymphosarcoma, yet not regress to the same degree. An outstanding example of the importance of the sensitivity of the tumor cells to radiation is found in the case of osteogenic sarcoma. Relatively large amounts of radiophosphorus may be concentrated in the neoplasm and in the surrounding bone, yet this radioresistant tumor responds poorly to radiophosphorus therapy. Tracer studies on the uptake of radiophosphorus by cancer of the human breast indicate that here, too, therapeutic amounts of radioactivity are not deposited in the neoplastic tissue (3).

It would appear unlikely, therefore, that therapeutic amounts of radiophosphorus can be localized in most growths of the soft tissues by depending entirely upon the utilization of ordinary cellular metabolic processes for its localization and concentration.

One of us (1) has attempted to modify the tissue phosphorus uptake in animals by dietary means. Though more efficient utilization could be obtained by upsetting the calcium balance, there was no significant alteration in the normal ratio of phosphorus uptake by the bone and soft tissue.

*This study was supported in part by a grant from The Commonwealth Fund.

Since the selective deposition of the radiophosphorus in cancers is not adequate to produce destruction of the tumor when dibasic sodium phosphate is administered orally or intravenously, studies were carried out to determine its distribution and concentration when injected directly into the tumor. Spontaneous mammary adenocarcinoma developing in the C57 strain of mice was transplanted into other animals of this strain. Radioactive disbasic sodium phosphate was injected directly into the tumor in one group of animals, and subcutaneously in a group of normal mice used as controls. Twenty-four hours later various tissues were removed, ashed, and analyzed for radiophosphorus in terms of grams administered and grams of tissue. The distribution is illustrated in Chart I. As will be noted, there was no significant difference in the uptake by the experimental animals and those in the control group. Bone still received a greater portion than any other organ, and the radiophosphorus present in the tumor was actually less than that in normal viscera of high phospholipid content, such as the liver and spleen. It is not surprising, therefore, that those animals that were injected and allowed to survive showed no evidence of tumor regression.

As a result of these observations, and from the experimental and clinical experience of others, we felt it highly improbable that one could anticipate any significant therapeutic effect on many types of cancer by reliance upon the use of radioactive dibasic sodium phosphate alone. However, it occurred to us that if the radiophosphorus could be injected into the tumor in an insoluble form so that it was not rapidly dissipated, and thus remained in direct apposition with the malignant cells for a considerable length of time, different results might be obtained. Radiophosphorus should be an excellent isotope for such therapy for several reasons. It emits beta rays with a maximum energy of 1.69 million electron volts, which are rather destructive to tissue. The range of action, approximately 0.7 cm., is adequate for the irradiation of tissues nearby, and the relatively rapid decay avoids delayed radiation effects. This rate of decay (14.3 day half life) is long enough, however, to permit action on several mitotic cycles.

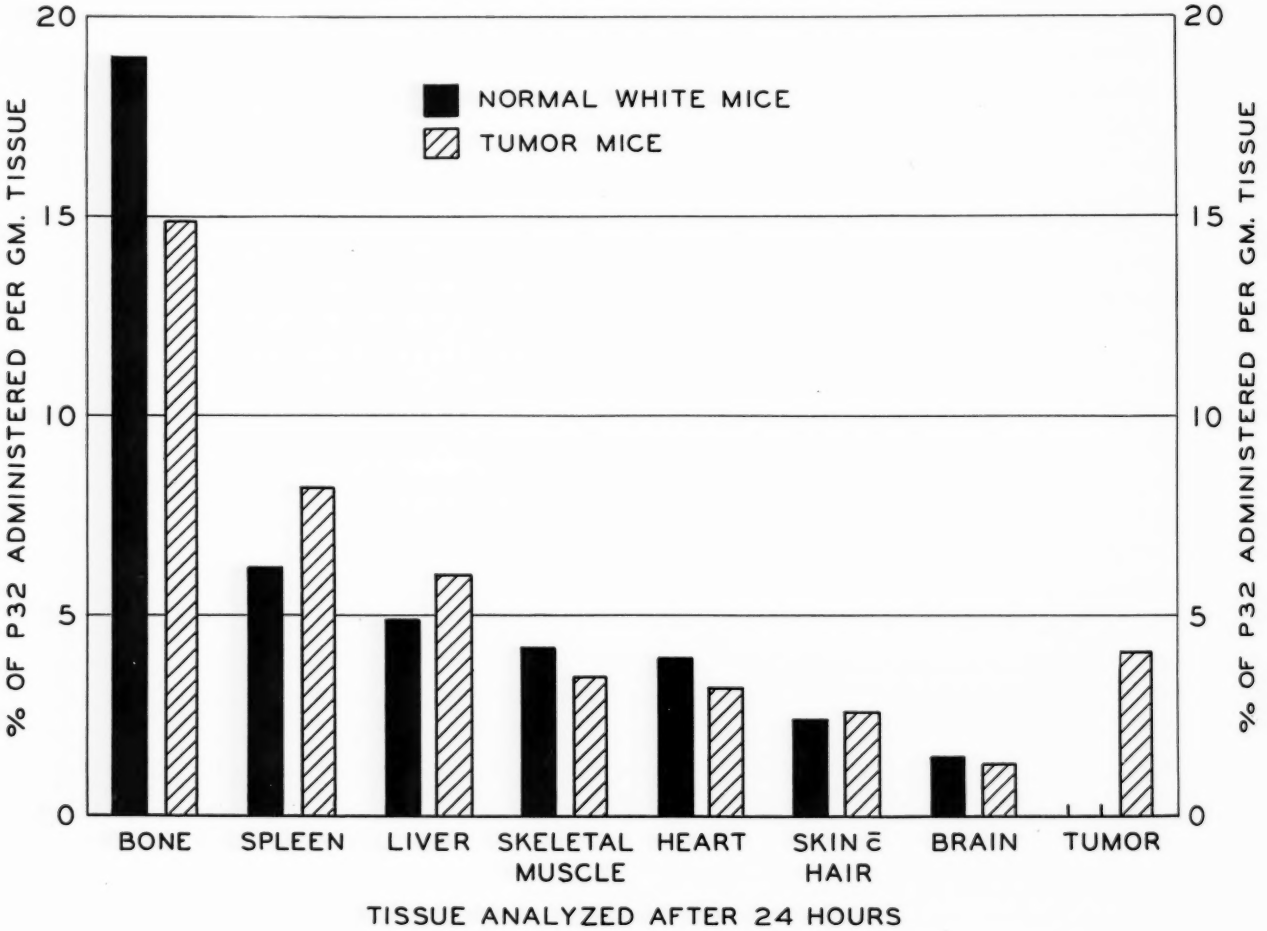

CHART I.—Amount of radiophosphorus remaining in various tissues 24 hours after radioactive disodium phosphate was injected directly into mammary adenocarcinoma in mice, as compared with its distribution in tissue following injection of a similar quantity with identical activity subcutaneously into normal mice.

A suitable insoluble radiophosphorus compound is chromium phosphate, which has been used previously by Jones, Wrobel, and Lyons (2) in the treatment of metastatic tumors of the liver in human patients. It is chemically inert, and can be made into a fine suspension. Radioactive chromium phosphate was therefore selected as a suitable compound.

Preparation of Radioactive Disodium Phosphate

Red phosphorus was bombarded with 12 million volt deuterons in the cyclotron of the Mallinckrodt Institute of Radiology, Washington University, St. Louis, with the resulting formation of the radioactive isotope P^{32}.[1] The radiophosphorus was converted into disodium phosphate, which, in turn, was made into isotonic solution. The radioactivity of this solution was determined by means of a Geiger counter, and it was used as the standard solution from which chromium phosphate was prepared.

Preparation of Chromium Phosphate[2]

1. Prepare 0.1 *N* solution of radioactive dibasic sodium phosphate (Na_2HPO_4).
2. Mix with an equal volume of 0.5 *N* chromium nitrate [$Cr(NO_3)_3$] and stir. Two hundred and fifty cubic centimeters of the phosphate solution will yield approximately 1.5 gm. of dried $CrPO_4$.
3. To the mixture add 1.0 *N* sodium acetate ($NaC_2H_3O_2$) to one-third of the volume of the phosphate solution.
4. Stir, centrifuge, and pour off supernatant fluid.
5. Add small amount of distilled water, stir up precipitate, and centrifuge.
6. Decant supernatant fluid, wash precipitate with distilled water, and centrifuge.
7. Pour off supernatant water and transfer precipitate to evaporating dish.
8. Dry precipitate and then heat on hot plate until the green precipitate changes to a dark grey-brown. The color change may be abrupt. The color may vary depending upon the amount of free chromium left from the washing of the precipitate. Heat until the whole mass has changed color throughout, but do not heat above 600° C. This may require several hours.
9. Transfer to a large serum bottle, the bottom of which is covered with glass beads about 3/16 inch in diameter, add sufficient isotonic saline to lubricate the beads and to make a thin paste of the $CrPO_4$.
10. Rotate the bottle as a ball mill for at least 12 hours at a speed where the beads cascade over each other. The original precipitate is chiefly $CrPO_4 \cdot 4H_2O$. The heat breaks up the crystal pattern, leaving $CrPO_4$ in a finely divided state, but lumped together in a seemingly amorphous mass. The glass beads in rotating separate the particles. Twelve hours of grinding will reduce the particle size to less than 1 micron in diameter. When taken from the rotator the $CrPO_4$ looks like clay. Upon further dilution with isotonic saline, the mud takes on definite characteristics of a suspension. Dilution to 15 mgm. per cc. with physiologic salt solution is sufficient to have the suspension last from 10 to 15 minutes after shaking. We have found this to be a satisfactory concentration for subcutaneous injections. Weaker dilutions give more stable suspensions. Following preparation of the suspension the material is sterilized in an autoclave and then kept in a refrigerator at 4° C. until ready for use. Injections may be made with a #24 hypodermic needle, and produce no more pain in human patients than a similar injection of physiologic salt solution.

Treatment of Tumor

The tumor used in this experiment was a transplantable spontaneous mammary adenocarcinoma of the C57 brown strain of mice. The trocar method of transplantation was used, and transplantation was generally successful. Various sizes of these transplanted tumors were treated.

At first local injections of chromium phosphate were tried by placing the material in the center of the tumor. This was soon given up because many of the animals died directly. Necropsies showed that death occurred because some of the injected material had been carried directly to the vessels of the heart. While such a result would probably not be obtained in larger animals with cardiac blood vessels of greater diameter, the method was unsatisfactory in mice. It occurred to us that the reason for such immediate transportation to the general circulation may be related to the larger size of the tumor capillaries and to an increase in the permeability of their walls that is known to exist. However, as much as 15 mgm. may be given intravenously to mice with no similar difficulty. It was felt that if the injections were made into the more or less normal soft tissue adjacent to the periphery of the tumor such embolisms in all probability would not occur. This was found to be true. It was noted that multiple small doses of chromium phosphate, in sufficient amounts to cover the entire

[1] The authors are indebted to Dr. Harry Fulbright and his staff at the cyclotron for supplying the radiophosphorus.

[2] (This method of preparation of chromium phosphate was kindly supplied us by Dr. H. B. Jones, of the Crocker Radiation Laboratory, University of California. He has used this material intravenously in the treatment of metastatic tumors of the liver. In a personal communication he states that he has also treated successfully a number of lymphoid tumors in mice by direct inoculation. It is unfortunate that his experiments were discontinued because of urgent war work.)

periphery of the tumor, could be injected safely. For every group of tumor mice receiving radioactive chromium phosphate, two control groups were used. One of these received no injections, and the tumor was allowed to progress spontaneously; the other received injections of inactive chromium phosphate in the same amounts and in relatively the same position about the tumor as in the group receiving the radioactive chromium phosphate. In all three groups of animals the tumors were the same age and of approximately the same size.

RESULTS

Death occurred in all control animals in from 6 to 8 weeks from the time of inoculation, and apparently was related to progressive increase in the size of the tumor. Those animals whose tumors were treated with radioactive chromium phosphate responded differently, according to the size of the growth and the amount of radioactive material used. It was found that the mice could tolerate less than 1 mc. at a single injection. About 0.1 mc. seemed to be the optimum dose for neoplasms less than 2 cm. in diame-

TABLE I

Mouse no.	Tumor age, days	Tumor size, cm.	No. daily injections	Amount $CrPO_4$, mc.	Life of animal	Results
1	14	1 x 1.5	12	0.733	40 days	Tumor regressed completely. Sacrificed for study.
2	14	1.5 x 1.5	12	0.573	38 "	" regressing. Died—radiation necrosis and infection.
3	14	1 x 1.5	12	0.736	4 mos.	" regressed completely. Sacrificed for study.
4	21	2.5 x 1.5	6	0.462	5 days	Died during injection.
5	21	1.7 x 1.5	8	0.810	7 "	" " "
6	21	3 x 2	4	0.417	3 "	" " "
7	17	1 x 1.3	8	0.811	22 "	Tumor regressed. Died—radiation necrosis and infection.
8	17	1 x 1.1	8	0.811	20 "	" " Sacrificed for study.
9	17	0.8 x 0.5	5	0.811	13 "	" regressing. Died—radiation necrosis and infection.
10	11	0.7 x 0.8	7	0.973	6 mos.	Living—tumor regressed completely.
11	11	0.9 x 1.2	5	1.027	3 "	Tumor regressed completely. Sacrificed for study.
12	11	1.1 x 1.4	7	1.298	22 days	" regressing. Died—radiation necrosis and infection.
13	11	1.1 x 1.6	1	0.108	0	Died during injection.
14	11	0.8 x 1.3	8	0.704	20 "	Sacrificed for study. Tumor regressing.
15	11	1 x 1.3	9	0.649	3 mos.	Tumor regressing. Sacrificed for study.
16	11	1 x 1.4	1		0	Died during injection.
17	14	1.2 x 2	3	0.850	5 days	Sacrificed for study.
18	14	1.4 x 1.5	2	0.328	1 day	Died during injection.
19	14	2 x 1.2	3	0.769	30 days	Tumor regressing. Died—radiation necrosis and infection.
20	14	1.5 x 1	3	0.506	6 mos.	Living.
21	14	1.3 x 1.8	1		0	Died during injection.
22	14	1.7 x 1.2	3	0.729	15 days	" Cause unknown.
23	14	1.7 x 1	3	0.546	13 "	Tumor regressing. Died—radiation necrosis and infection.
24	14	2 x 1.5	3	0.668	13 "	" " " " " " "
25	10	1 x 0.5	8	0.114	30 "	Tumor regressed completely. Sacrificed for study.
26	10	1.5 x 0.5	8	0.200	6 mos.	Living. Tumor regressed completely.
27	10	1.5 x 1	8	0.280	2 "	Died. Insufficient radiation.
28	10	0.5 x 0.7	8	0.177	6 "	Living. Tumor regressed completely.
29	10	0.5 x 0.7	8	0.140	3 "	Tumor regressed completely. Raised litter.
30	10	0.5 x 0.4	8	0.132	5 "	" " " Sacrificed for study.
31	10	1.5 x 1.5	8	0.372	2 "	Died. Insufficient radiation.
32	30	2.5 x 3.5	2	0.082	34 days	" Tumor too large.
33	30	2.5 x 3	2	0.082	7 "	" " " "
34	30	2.5 x 4	2	0.082	20 "	" " " "
35	30	2.5 x 2	2	0.082	34 "	" " " "
36	30	2 x 1	2	0.082		Living. Tumor regressed completely.
37	30	2 x 1	2	0.082		" " " "
38	30	2 x 1	2	0.082		" " " "
39	30	2 x 1	2	0.082		" " " "
40	30	2 x 1	2	0.082		" " " "
41	30	2 x 1	2	0.082	8 wks.	Tumor regressed completely. Sacrificed for study.
42	30	0.5 x 0.7	2	0.050		Living. Tumor regressed completely.
43	30	2.5 x 2.5	2	0.082	13 days	Died. Tumor too large.
44	30	2 x 2	2	0.082		Living. Tumor regressed completely.
45	30	2.5 x 3.5	2	0.082	34 "	Died. Tumor too large.
46	30	2.5 x 3.5	2	0.082	34 "	" " " "
47	30	3.5 x 2.5	2	0.082	34 "	" " " "

ter; those over 2.5 × 2.5 cm. in size were not cured, and their bearers died apparently from infection and toxemia associated with necrosis of the tumor. Roughly speaking, those experiments in which the mass of the growth was equivalent to more than one-fifth of the body weight, ended in the death of the animal, apparently from massive tissue breakdown. Smaller tumors, where an adequate but not excessive amount of radioactive material was used, disappeared completely in every instance (Table I).

DISCUSSION

In the 47 groups of animals studied, various doses of radiophosphorus were used and different methods of injection tried. The response of tumors that were less than one-fifth the weight of the animal was entirely satisfactory in all but 2 cases; in these it was felt that not enough radiation had been given in the initial series of treatments. Additional radiophosphorus was not administered to these animals, even after it was obvious that the tumor response would

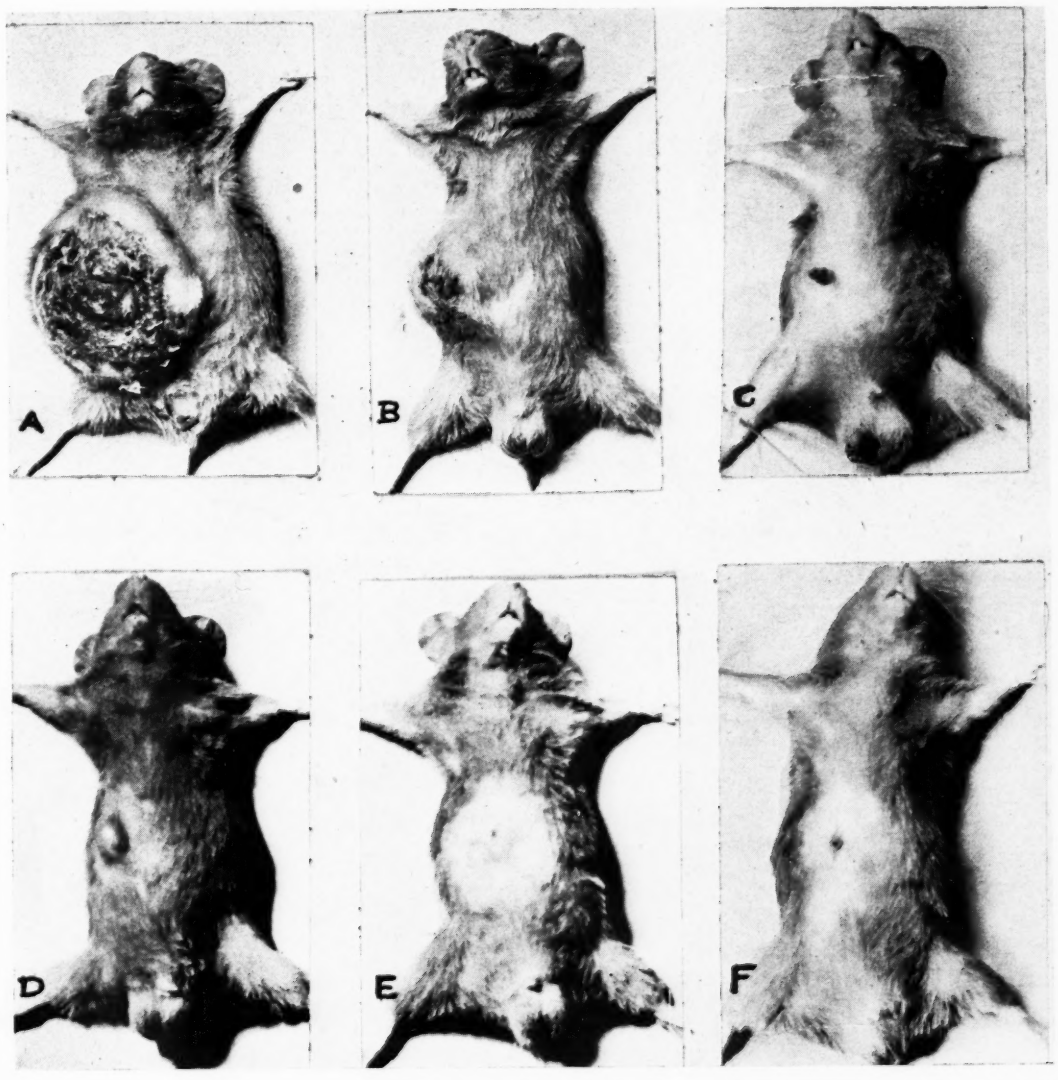

FIG. 1.—A. Untreated mammary tumor 8 weeks after transplantation. Mice with growths of this size generally died of toxemia during treatment, apparently from the absorption of broken down tissue. B. Untreated mammary tumor, approximately 3 weeks after transplantation. C. Growth was of similar size to tumor shown in B when treatment was started. Treatment for 7 days, during which time 0.646 mc. of radioactive chromium phosphate was injected. Note small area of ulceration, probably due to radiation. D. Mammary tumor 7 days after transplantation. E. Tumor of similar size to growth in D when treatment was started. Tumor age, 9 days. Treatment for 8 days, during which 0.144 mc. of radioactive chromium phosphate was injected. Photograph taken 4 weeks after treatment ceased. F. Tumor somewhat larger than that shown in D, being 15 days old when treatment was begun. Treated for 12 days, during which 0.763 mc. of radioactive chromium phosphate was injected.

be unsatisfactory. Several other mice died from infection while their neoplasms were regressing satisfactorily. One of the treated animals bore a litter of young 6 months after treatment. In no instance where satisfactory regression was observed was there a recurrence of the tumor. No case of spontaneous regression was observed in either set of controls. In tumors that regressed revealed no residual tumor cells in any of the cases sacrificed for study. Fig. 2C illustrates such a site; there is still a considerable accumulation of the chromium phosphate. The overlying skin shows hyperplasia of the epithelial layer, with absence of hair follicles. Fig. 2D, which was taken from the edge of the deposit of chromium phosphate,

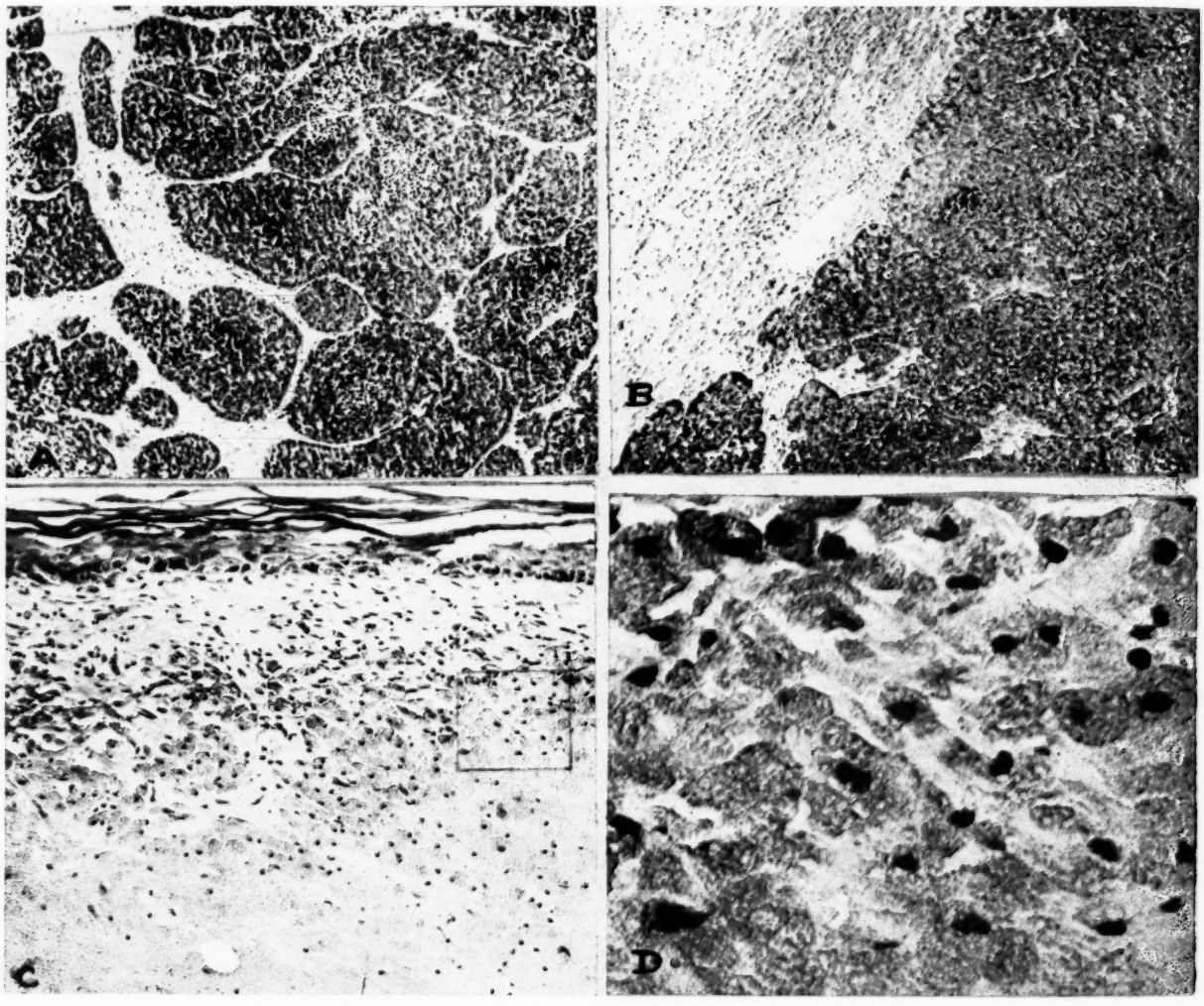

FIG. 2.—A. Adenocarcinoma of mamma from untreated control. Mag. × 107.5. B. Adenocarcinoma of control treated with inactive chromium phosphate. This may be seen at upper left of illustration, chiefly in phagocytes. Mag. × 107.5. C. Section from site of adenocarcinoma treated with radioactive chromium phosphate. Note absence of cancer cells, beginning fibrosis around remaining chromium phosphate, and appearance of epidermis. Hair follicles absent, and epidermis hyperplastic. Mag. × 104. D. Enlargement from Fig. 2C. Chromium phosphate particles phagocytosed by wandering cells and producing nuclear degeneration. Outlines of cells already destroyed may be seen. Mag. × 566.5.

several instances an ulcer persisted over the area for several weeks after regression of the tumor. This was interpreted as the result of too intense radiation.

Examples of the gross appearance of the neoplasms employed may be seen in Fig. 1. Fig. 2A illustrates the microscopic appearance of the tumor used, and Fig. 2B the control that was treated with inactive chromium phosphate. The material may be seen as a grey intracellular substance in the upper left portion of the photograph. Histologic examination of the sites of shows the remains of many phagocytes that engulfed the material in their cytoplasm and were then destroyed by it. In other cells the nuclei are hyperchromatic, and obviously badly damaged.

Assay of various organs of the treated animals for radioactivity showed only traces of the substance in the bone marrow, liver, and spleen, but there were often considerable amounts in the regional lymph nodes, with definite damage resulting, as may be seen from the edema and atrophy (Fig. 3A). Fig. 3B

illustrates chromium phosphate in the cytoplasm of the reticulum cells of the same node, some of which show nuclear damage. The practical significance of the distribution of chromium phosphate to the regional lymph nodes is still to be determined.

Although they are not included in this report, studies have been carried out on the action of radioactive chromium phosphate on transplanted squamous cell carcinoma, melanosarcoma, and fibrosarcoma in mice. In these tumors, also, regression has been noted.

The amount of radiation needed to produce regression in melanosarcoma and fibrosarcoma is much larger than that required to achieve excellent regression of the mammary tumors. This is to be expected, since the former have been shown to be extremely resistant to roentgen and gamma ray therapy while the

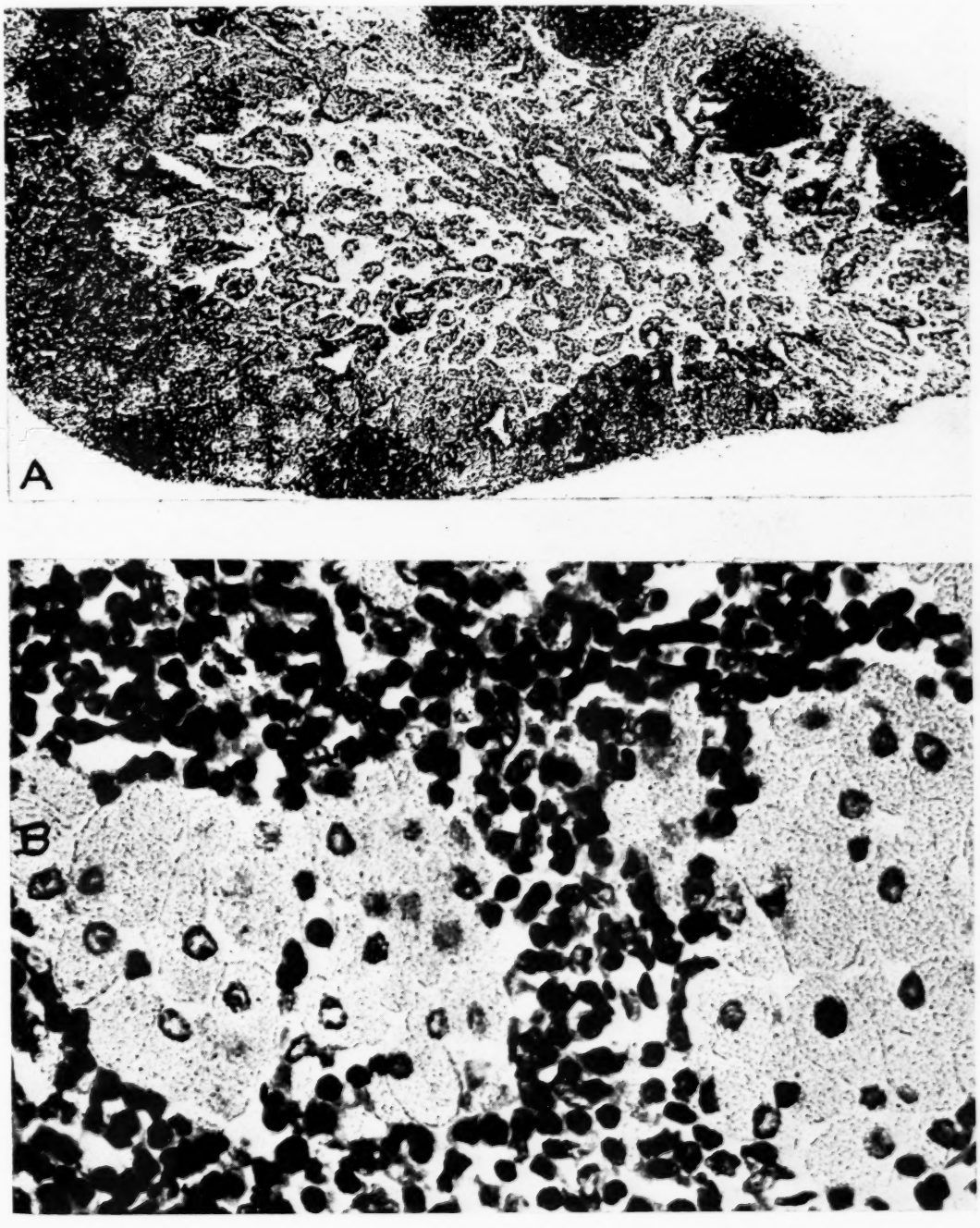

FIG. 3.—A. Axillary lymph node from mouse treated with radioactive chromium phosphate. Node drained area in which cancer was located. Note atrophy, edema, and chromium phosphate in reticuloendothelial cells. Mag. × 75. B. Enlargement from Fig. 3A. Reticuloendothelial cells containing chromium phosphate. Size of particles well illustrated. Nuclear damage evident, but activity of these particular particles when section was taken was apparently slight. Mag. × 780.

mammary tumor used in these experiments is an unusually sensitive one.

Clinical studies now being carried on will be reported upon at a later date.

CONCLUSION

Chromium phosphate, an insoluble salt of radiophosphorus, was injected in saline suspension around the periphery of transplanted spontaneous mammary adenocarcinomas in the C57 strain of mice, with resulting regression of the tumor. These growths can be completely and safely destroyed by this method of interstitial radiation. Where the neoplasms are larger than one-fifth the body weight of the animal, fatal toxemia often results from the absorption of broken down tumor tissue. Most of the radioactive material apparently remains at the site of injection although a small portion is carried to the regional lymph nodes, where its action can be noted. There is no microscopic evidence of distant parenchymatous damage.

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4. Low-Beer, B. V. A., Lawrence, J. H., and Stone, R. S. The Therapeutic Use of Artificially Produced Radioactive Substances. Radiophosphorus, Radiostrontium, Radioiodine, with Special Reference to Leukemia and Allied Diseases. Radiology, **39**:573-597. 1942.

Some Synonyms for "Marked"

An Editorial

Large	Striking
Great	Extensive
Signal	Noticeable
Strong	Noteworthy
Intense	Pronounced
Unusual	Significant
Decided	Conspicuous
Extreme	Substantial
Notable	Appreciable
Definite	Considerable
Distinct	Extraordinary

William H. Woglom

Abstracts

Reports of Research

Extrinsic Factors that Influence Carcinogenesis. Rusch, H. P. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Physiol. Rev.*, **24**:177-204. 1944.

This article reviews the extensive literature on the effects of the following groups of factors on experimental carcinogenesis: (1) Chemical agents, such as solvents, fats and oils, irritants, and miscellaneous chemicals, applied directly to the region of carcinogenesis. (2) Physical agents such as radiant energy and various forms of mechanical and thermal injury. (3) Combinations of carcinogens with each other, with x-rays, with ultraviolet radiation, or with viruses. (4) Dietary factors including various fats, vitamins, proteins, amino acids, tissue extracts, and calories as such. (5) Miscellaneous factors such as organic peroxides, growth-inhibiting extracts from human urine, and the milk agent in mouse mammary cancer.—R. B.

Präneoplasie und Regeneration. Experimentelle Untersuchungen am Kaninchen. [Preneoplasia and Regeneration. Experimental Investigations on Rabbits.] Fritzsche, H. [Zurich Univ., Zurich, Switzerland] *Ztschr. f. Krebsforsch.*, **54**:77-111. 1943. From abstr. in *Chem. Zentralbl.*, **II**:1636. 1943.

Confirmation of the observations of MacKenzie and Rous (*J. Exper. Med.*, **73**:391. 1941; abstr. in *Cancer Research*, **2**:303. 1942) on the production [by tarring] of a latent neoplastic state.—M. H. P.

Comparison of Some Carcinogenic Hydrocarbons with Reference to Their Activity. Roussy, G., Guérin, M., and Guérin, P. [Inst. du Cancer, Paris Univ., Paris, France] *Compt. rend. Soc. de biol.*, **136**:374-375. 1942. From abstr. in *Chem. Zentralbl.*, **II**:2294. 1943.

Methylcholanthrene produced about the same number of tumors as did benzpyrene, upon subcutaneous injection into rats and mice in doses of 1 or 0.1 mgm. of hydrocarbon in 1 cc. of olive oil. The former compound was somewhat more effective than the latter in mice, and the reverse was the case in rats. Dibenzanthracene was appreciably less active than methylcholanthrene or benzpyrene in both species.—M. H. P.

Connective Tissue Neoplasms Produced in Dogs by Methylcholanthrene. Mulligan, R. M. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Proc. Soc. Exper. Biol. & Med.*, **57**:134-135. 1944.

A male mongrel dog, about 3 years old, weighing 9.5 kgm., received subfascially in the left flank 50 mgm. of methylcholanthrene in a mixture of 3 cc. each of benzene and paraffin. At the end of 297 days a nodular tumor in direct continuity with the injected material was found. Autopsy on the 327th day disclosed a neoplasm of 6 nodules from 10 to 22 mm. in diameter. The microscopic diagnosis was connective tissue neoplasm with the histological characteristics of an immature fibrosarcoma. No metastases were found.

A male mongrel about 1½ years old, weight 8 kgm., was similarly injected in the left flank with 40 mgm. of methylcholanthrene. On the 361st day, 100 mgm. of methylcholanthrene in a gelatin capsule was implanted subfascially in the right side of the thorax. On the 735th day, a nodule directly continuous with the first methylcholanthrene implant was found; autopsy on the 791st day revealed a 23 mm., firm, white tumor along the lateral border of the left rectus muscle. The diagnosis was connective tissue neoplasm with the histologic characteristics of a mature fibrosarcoma. No metastases were found. At the locus of the second methylcholanthrene implant there was a 1.5 mm. nodule but no neoplasm. No neoplasms occurred in 6 other dogs receiving injections of 30 to 100 mgm. of methylcholanthrene.—M. B.

The Response of the Central Nervous System of the Rat to Methylcholanthrene. I. The Induction of Tumors Derived from Nervous Tissue. Russell, W. O. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research*, **5**:140-151. 1945.

Intracranial tumors were produced in 21 of 42 albino rats by means of pellets of methylcholanthrene in 30% concentration fused with chemically pure cholesterol, implanted in the right cerebral hemisphere. Fourteen of the 21 rats developed neoplasms derived from nervous tissues, and 10 developed fibrosarcomas; 3 animals had both types. Tumors of the following types, derived from nervous tissue, were described: 6 cases of spongioblastoma multiforme, 2 astrocytomas, 1 oligodendroglioma, and 2 tumors that were composed chiefly of large numbers of fairly well differentiated nerve cells. The 2 last named tumors are the first instances reported of induced neoplasia of nerve cells. It was not possible to classify definitely 3 of the tumors derived from nervous tissue. The tumors thus derived were soft and infiltrating, frequently contained foci of hemorrhage, and generally were poorly demarcated from the surrounding nervous tissue. They formed a characteristic group easily differentiated macroscopically from the fibrosarcomas, which were sharply circumscribed and firm, and rarely contained hemorrhagic foci. These experiments demonstrated that the cells of the central ner-

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vous system of rats respond to carcinogenic stimulation as readily as those of the mouse if a high concentration of carcinogen is kept in contact with the nervous tissue for a sufficiently long period of time.—Author's abstract.

The Response of the Central Nervous System of the Rat to Methylcholanthrene. II. The Effect of a Diet Deficient in Thiamine and Riboflavin on the Induction of Tumors Derived from Nervous Tissue. Russell, W. O. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research,* **5**:152-156. 1945.

It was demonstrated in these experiments that the periodic removal of thiamine and riboflavin from the diet of white rats with intracerebral pellets of methylcholanthrene produced a significant reduction in the length of the induction period for tumors derived from nervous tissue. Their average induction time was 230 days for the rats given a deficient diet, and 372 days for those fed the control diet. The incidence of tumors derived from nervous tissue was the same in the animals receiving the deficient and the adequate diets. The deficiency had no effect upon either the incidence or the length of the induction period for the tumors derived from connective tissue. The author suggested that the altered metabolism of the cells of the nervous system resulting from lack of thiamine and riboflavin caused the cells to respond more readily to the carcinogen. It is not possible to say which deficiency was responsible for the altered susceptibility of the nervous tissue.—Author's abstract.

Attempts to Induce Stomach Tumors. IV. The Effects of (a) Cholesteryl Esters Heated to 300° C., and (b) Cholesterol Heated to 430° C. Kirby, A. H. M. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] *Cancer Research,* **5**:129-134. 1945.

Rats given an adequate basal diet developed no significant lesions of the stomach nor tumors of any part of the body when fed with the products of heating either cholesteryl stearate or palmitate to 300° C. for half an hour at a 25 mgm. per day level for 15 months, and allowed to live out their natural span. Similar rats, fed with the same basal diet plus 25 mgm. daily of the products of heating cholesterol for half an hour to 430° C., showed only a slight degree of hyperkeratosis of the forestomach and no tumors of the glandular zone, nor any tumors in any other part of the body. The pyrolysis of cholesterol at 430° C. has been shown to break the molecule at C_{17}, yielding methyl*iso*hexylketone and the Diels hydrocarbon, $C_{18}H_{16}$, which may be carcinogenic. The fluorescence of the pyrolysis product is not yet accounted for.—Author's summary.

Tumors Induced with Heated Cholesterol. Beck, S., Kirby, A. H. M., and Peacock, P. R. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] *Cancer Research,* **5**:135-139. 1945.

Heated fats and heated cholesterol have been under suspicion for some years as possible etiological factors in gastric cancer. Experimental proof has been unconvincing as far as lesions of the stomach are concerned, but it must be remembered that no feeding experiments with any substance have so far resulted in undisputed adenocarcinoma of the stomach in animals. As chemical carcinogens are not restricted to 1 type of tissue, the demonstration of carcinogenic properties in heated fats and heated cholesterol means that these substances may be of etiological importance in regard to gastric cancer.

In the present experiments, (a) cholesterol heated to 270 to 300° C. for half an hour, (b) the residue from (a) after the removal of dicholesteryl ether and Δ 4-cholestenone, (c) cholesteryl stearate heated to 300° C. for half an hour, (d) cholesteryl palmitate similarly treated, and (e) cholesterol heated to 430° C. for half an hour have been tested by painting or injection in stock mice. Substances (a) and (b) induced spindle-cell sarcomas in the walls of the injection cyst; substance (e) induced papillomas, 2 of which regressed. Other tumors that occurred during these experiments at sites remote from the applications of these products have been recorded, but it is not possible to assess their significance. The conclusion is drawn that heated cholesterol, as well as heated cooking fats, is a potential source of carcinogens, which are very weak by comparison with pure carcinogenic hydrocarbons, such as methylcholanthrene, but possibly of more practical significance owing to their frequent occurrence in cooked foods.—Authors' abstract.

Testicular Tumors in the Teleost (*Xiphophorus helleri*) Receiving Sesame Oil. Li, M. H., and Baldwin, F. M. [Univ. of South. California, Los Angeles, Calif.] *Proc. Soc. Exper. Biol. & Med.,* **57**:165-167. 1944.

Sesame oil, which is often used as a vehicle for fat-soluble hormones given subcutaneously, produced tumors of the interstitial cells of the testis in 3 of 40 adult male teleosts (*Xiphorus helleri*). Two of the tumors were malignant. In one series of experiments, 8 males served as untreated controls, and 8 received 4 to 6 weekly doses of 0.02 cc. of sesame oil retroperitoneally. In a second series comprising 44 adult males, 12 served as untreated controls, and 32 were injected with 0.02 cc. of sesame oil at intervals of 15 days. Six of them each received 27 or more injections.

The first tumor, occurring in a fish that had received 6 injections, replaced a large portion of the sperm tubules. The tumor cells, identified as interstitial cells, were polygonal with single, nearly rounded, vesicular nuclei. Many of these cells were vacuolated; mitoses and blood vessels were rare. In the second series a malignant tumor of the testis, similar to the one described above, occurred in a fish after 23 injections. Mitoses were rare, and many cells showed foamy cytoplasm or necrosis. In a third fish, which had received 23 injections, 7 small nodules of interstitial cell hyperplasia were discovered. These nodules comprised many foamy tumor cells. The control fish exhibited no neoplasia.—M. B.

The Determination of *p*-Dimethylaminoazobenzene, *p*-Monomethylaminoazobenzene, and *p*-Aminoazobenzene in Tissue. Miller, J. A., and Baumann, C. A. [Coll. of Agric., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **5**:157-161. 1945.

A quantitative method has been devised for the simultaneous determination of *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, and *p*-aminoazobenzene in rat tissues. The dyes are extracted from the unsaponifiable matter with 7 *N* HCl, and after neutralization are returned to petroleum ether and passed through adsorption columns of suitably prepared alumina. The color intensity

of the eluted fractions is then read in 7 *N* HCl. The method is suitable for the recovery of as little as 1.0 μgm. of each dye from tissue.—Authors' abstract.

On the Methylation and Demethylation of Certain Carcinogenic Azo Dyes in the Rat. MILLER, J. A., MILLER, E. C., and BAUMANN, C. A. [Coll. of Agric., Univ. of Wisconsin, Madison, Wis.] *Cancer Research,* **5**:162-168. 1945.

The tissues of rats fed *p*-dimethylaminoazobenzene contained not only the dye itself, but its demethylated derivatives, *p*-monomethylaminoazobenzene and *p*-aminoazobenzene. The amounts of these 3 dyes found in the body accounted for approximately 8% of the dimethyl compound ingested during the previous 24 hours. Most of the dye was located in the red blood cells in the form of the completely demethylated derivative, *p*-aminoazobenzene. When *p*-monomethylaminoazobenzene was fed to rats, the liver contained both *p*-aminoazobenzene and *p*-dimethylaminoazobenzene in addition to the dye fed; the blood contained only *p*-aminoazobenzene. The distribution of the 3 dyes was quantitatively the same whether the monomethyl or dimethyl derivative was fed. The presence of *p*-dimethylaminoazobenzene in the livers of rats fed the monomethyl compound indicates that the rat is capable of methylating the latter substance. When either the monomethyl or dimethyl compound was fed or injected intraperitoneally the contents of the intestinal tract contained both the dye administered and *p*-aminoazobenzene with the latter dye predominant. The distribution of the 3 dyes in the tissues was essentially the same whether the monomethyl or dimethyl compound was fed or injected intraperitoneally. When feeding of the dye was stopped, the concentration of all 3 forms in the tissues decreased rapidly. When *p*-aminoazobenzene was fed to rats, the only form of dye found in the tissues was *p*-aminoazobenzene itself.

These findings demonstrate that *p*-dimethylaminoazobenzene is demethylated *in vivo* prior to reduction at the azo linkage. The high levels of *p*-aminoazobenzene in the body and the rapid rate of disappearance of the 3 dyes therefrom indicate that demethylation prior to cleavage constitutes a major pathway in the metabolism of the methylated dyes. It is possible that the metabolism of *p*-dimethylaminoazobenzene follows this pathway to such an extent as to preclude the formation of N,N-dimethyl-*p*-phenylenediamine, the alleged carcinogen according to one current theory.—Authors' abstract.

Effect of Feeding Dried Milk on Production of Liver Cancer by *p*-Dimethylaminoazobenzene. SUGIURA, K. [Memorial Hosp., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.,* **57**:231-234. 1944.

It has previously been shown that the incidence of liver cancer produced by *o*-aminoazobenzene or *p*-dimethylaminoazobenzene (butter yellow) is reduced when the basal diet consists of wheat, rye, or millet seed instead of rice. Also, the addition of yeast or liver to a rice diet increases resistance to this type of tumor formation, and liver cancer will not be produced by *p*-dimethylaminoazobenzene on a rice diet supplemented with either casein and riboflavin, casein and B vitamins, or cystine and choline.

In the present investigation, the feeding of dried whole milk (klim), chosen because of its high casein and riboflavin content, was found to inhibit partially the development of liver cancer in rats fed *p*-dimethylaminoazobenzene. Three groups of 20 rats each (Sherman stock, weight 125 to 150 gm.) were kept on a basal diet of unpolished rice mixed with 6, 15, and 20% dried whole milk respectively. Fifty rats kept on unpolished rice alone served as controls. All animals received 3% *p*-dimethylaminoazobenzene in cottonseed oil, in the proportion of 20 cc. of solution per kgm. of ration, and a small amount of carrot daily. The diets were fed for about 200 days; all surviving animals were then killed. Among the rats fed 20% dried whole milk, 50% had grossly and histologically normal livers; the remainder showed livers with numerous tumor nodules. The group fed 15% whole milk had 60%, and that fed 6% had 90% liver cancer. The controls all developed liver cancer and in shorter time than the experimental groups.

The effect was studied also of maintaining rats on a *p*-dimethylaminoazobenzene-rice diet for definite periods, then removing the carcinogen and feeding unpolished rice plus 15% dried whole milk. Of 20 rats given the carcinogen and rice for 62 days, followed by the rice-whole milk diet for 113 to 168 days, 90% showed normal livers. In contrast, among rats fed the carcinogen and rice for the same period, followed by rice alone, only 12% had normal livers. Mice fed the carcinogen for more than 88 days almost all developed liver cancer. When the tumor was once established, its growth was not affected even though dried whole milk was fed for long periods after the feeding of *p*-dimethylaminoazobenzene was discontinued.—M. B.

The Action of Carcinogenic Compounds and Related Substances on Enzymes. FEIGENBAUM, J. [Cancer Research Lab., Hebrew Univ., Jerusalem, Palestine] *Exper. Med. & Surg.,* **2**:304-310. 1944.

The action of pancreatic protease, specially prepared from pancreatin, on casein or gelatin *in vitro* was slightly inhibited by Na 1,2,5,6-dibenzanthracene-9,10-$\alpha\beta$-endosuccinate, 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, methylcholanthrene, anthracene, pyrene, phenanthrene, cholic and desoxycholic acids and their Na salts, Na taurocholate, cholesterol, estrone, ergosterol, and diethylstilbestrol dipropionate, but not by Na succinate. Pancreatic amylase was activated by the anions of the Na salts mentioned above, but not by the nonelectrolytic compounds. The effects of the compounds on the enzymes showed no correlation with their carcinogenic activity. Pancreatic lipase was not affected by any of the carcinogens whether they were kept in the dark or irradiated by the sun. Similarly, the carcinogenic hydrocarbons did not affect papain, pepsin, or maltase, sucrase, and phosphatase from taka-diastase. A method is described for the preparation of aqueous colloidal solutions of carcinogenic hydrocarbons and related compounds.—M. H. P.

Wiederherstellung der normalen d-Aminosäure-oxydasewirksamkeit durch Tumorentfernung. [Restoration of Normal d-Amino Acid Oxidase Activity by Tumor Removal.] WESTPHAL, U. [Inst. f. Physiol. u. Wehrchem., Militärärztl. Akad., Berlin, Germany] *Naturwissensch.,* **31**:117-118. 1943.

The author previously showed (*Ztschr. f. physiol.*

Chem., **276**:191. 1942; abstr. in *Cancer Research*, **4**:715. 1944) that the *d*-amino acid oxidase activity of the organism, as exhibited in liver and kidney extracts, was reduced in rats by the presence of transplanted Walker tumor. He now reports that when such tumors are completely extirpated 2 to 4 weeks after inoculation, in the absence of metastasis or recurrence the *d*-amino acid oxidase content of the liver increases, becoming normal by 10 to 12 days after tumor extirpation. A smaller increase in liver *d*-amino acid oxidase content is obtained when the tumors are incompletely removed. Apparently the amount of tumor tissue present determines the extent of reduction of the liver *d*-amino acid oxidase content.—M. H. P.

The Effect of Roentgen Radiation on the Thymonucleic Acid Content of Transplantable Mammary Carcinomas. Stowell, R. E. [Washington Univ. Sch. of Med., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research*, **5**:169-178. 1945.

The relative content of thymonucleic acid in transplantable mammary carcinomas in rats and mice was measured by the Feulgen reaction for thymonucleic acid and a special microphotometric apparatus. The tumor transplants on one side of the animal were treated with dosages of radiation that varied up to 4,000 r, while the control tumors on the other side of the animal were protected by lead shielding. The 6 tumors in rats that received 4,000 r all showed a statistically significant decrease in the amount of thymonucleic acid per unit volume, and 2 tumors showed a significant decrease in the amount per cell. The results of irradiation with 2,000 r were variable and not significant. In 9 of the 16 irradiated mouse tumors, the decrease in thymonucleic acid per unit volume was statistically significant, and in 4 of these the decrease per cell was significant. The results of these experiments support the hypothesis that roentgen radiation may alter the molecular structure of vital substances in the nucleus, producing a disturbance of the nucleoproteins as evidenced by an increase in ribonucleic acid and a decrease in desoxyribonucleic acid.—Author's abstract.

Tumor-bearing Chicks Hatched from Eggs Inoculated with Mouse Carcinoma. King, J. T., Ball, Z. B., and Menefee, E. C. [Univ. of Minnesota, Minneapolis, Minn.] *Proc. Soc. Exper. Biol. & Med.*, **57**:3-4. 1944.

Fertile eggs were inoculated on the fifth day of incubation with 0.1 to 0.6 cc. of Tyrode-solution suspension of mammary carcinoma of strain A mice. Nine chicks hatched and lived. These were killed with ether and examined at 24, 48, or 72 hours after hatching. Tumors were found in 7 of the 9. The tumor-bearing yolk sacs were in the normal position in the abdominal cavity but were more than normally adherent to the abdominal wall at the umbilical area. Most of the tumors were found near the point of contact, bulging into the yolk sac. They varied in size from 1 to 10 mm. or more. Grossly the tumors were firm, fleshy masses; microscopically most of them showed changes suggesting damage by the host.—M. B.

The Milk Agent in Spontaneous Mammary Carcinoma. Barnum, C. P., Ball, Z. B., Bittner, J. J., and Visscher, M. B. [Univ. of Minnesota, Minneapolis, Minn.] *Science*, **100**:575-576. 1944.

Mouse mammary cancer tissue was homogenized with distilled water, buffers, or saline; then lyophilized or cleared by centrifugation and treated in various ways before being injected into test mice 4 to 10 weeks of age. These mice were mostly hybrids of C57 black females and A strain males, or backcrosses of these hybrids to A males. The results of the tests show that the milk agent is destroyed by exposure to temperatures of 60° C. or higher for 1 hour, that it is stable at pH values between 5.0 and 10.2 but probably not at pH 4.5, that it is not inactivated by acetone or petroleum ether and not appreciably soluble in these solvents, and that it is partially precipitated by salmine at pH values of 5.5 and 6.8.—R. B.

Role of Heredity in Tumor Development. Heston, W. E. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:161-171. 1944.

A general consideration of the role of heredity in the development of neoplasms in experimental animals. The concept of the inheritance of a degree of cancer susceptibility vs. alternative expression of the character, the importance of the interaction of genic inheritance and environment, and the multiple-factor aspect of cancer inheritance are discussed in particular. A bibliography of 54 references is appended.—R. A. H.

Influence of Carcinogenic Hydrocarbon upon Transplanted Leukemia. Stamer, S., and Engelbreth-Holm, J. [Univ. Inst. Path. Anat., and Cancer Research Lab. of Danish Anti-Cancer League, Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **20**:360-371. 1943.

Transplanted leukemia was cured in all of 5 mice by 5 intravenous injections, each containing 1.5 mgm. of 9,10-dimethyl-1,2-benzanthracene in aqueous suspension, given in 1 to 9 days after the transplantation. Mice that received 2 to 3 injections lived twice as long after transplantation as did the untreated controls, but finally died with fully developed leukemia. All the controls died within 20 to 30 days. The suspension produced no tumors on intravenous injection, although on subcutaneous injection of low doses it was as actively carcinogenic as 9,10-dimethyl-1,2-benzanthracene in olive oil. The aqueous suspension was at first prepared by Boyland's method of dropping an acetone solution into hot water or hot gelatin solution to make a final concentration of 0.05%. A 0.5% suspension, with which the 5 cures mentioned above were apparently obtained, was subsequently prepared by using CO_2-free water, covering the aqueous surface with a layer of oil or paraffin saturated with the hydrocarbon to protect the suspended hydrocarbon against the precipitating effect of atmospheric CO_2, and removing the acetone by aeration with CO_2-free air.—M. H. P.

Effect on Leukemia in Mice from Lowering of the Body Temperature. Engelbreth-Holm, J., and Eltorm, H. [Univ. Inst. Path. Anat., and Cancer Research Lab. of Danish Anti-Cancer League, Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **20**:346-359. 1943.

Some temporary subsidence of leukemic infiltrations,

and prolongation of life, but no cures, were obtained when mice with spontaneous leukemia were starved and then chilled for 5 to more than 24 hours in a room at 6 to 8° or at 18° C. in order to produce a body temperature of 15 to 25° C. The leukemic cells showed degenerative response to cold therapy, but the normal cells did not. The degenerated cells in the lymph nodes disappeared within 3 to 4 hours after the end of the cold treatment, and the leukemic infiltrations subsided in 6 of 18 animals that were treated for more than 15 hours with a lowering of body temperature to below 25° C. The beneficial effect was most pronounced 3 to 5 days after treatment; subsequently the leukemic processes reappeared, and in 1 to 2 weeks there was a fully developed leukemia to which the animals succumbed. Of 140 mice treated by cold for spontaneous leukemia, 50 died from the exposure, 50 died of intercurrent diseases or were ruled out for other reasons, and 40 survived the treatment but died later. Photomicrographs are included.—M. H. P.

Tissue Culture Studies of Cytoplasmic Inclusion Bodies in Lymph Nodes of Hodgkin's Disease. Grand, C. G. [Washington Square Coll. of Arts and Sc., New York Univ., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **56**:229-230. 1944.

Lymph nodes from 35 cases of early and late stages of Hodgkin's disease were obtained surgically. As controls, lymph nodes were used from several cases each of mastectomy, lymphosarcoma, leukemia, metastatic carcinoma, and lymphadenitis. The nodes were cut into explants about 1 cu. mm. in size, from which tissue cultures were prepared. The medium consisted of a mixture of fowl plasma, human serum (in some cases from patients with advanced Hodgkin's disease), and chick embryo extract. After 48 to 72 hours incubation, there appeared on the periphery of the explants large multinucleated giant cells identified as Reed-Sternberg cells. These were found in nodes from every case of true Hodgkin's disease and were absent from the control cultures. Since virus infection has been considered a cause of Hodgkin's disease, brilliant cresyl blue 1:50,000 (a vital dye for virus cell inclusions) was used to stain the explants. Within 15 minutes after exposure to the dye, granules in the central body of the Reed-Sternberg cells stained from red to purple. These granules did not stain with Janus B green 1:20,000. Fibrocytes, macrophages, and lymphocytes in the same cultures regularly exhibited cytoplasmic inclusions that stained similarly with brilliant cresyl blue; cells of the same types from control lymph nodes did not show such inclusions.

When fragments of normal lymph nodes were grown in a culture medium containing noncellular extracts of cultured nodes of Hodgkin's disease, and were stained with brilliant cresyl blue, the lymphocytes and macrophages of these normal nodes showed cell inclusions within vacuoles of the kind found in cultures of nodes of Hodgkin's disease.

Supernatant extract from cultures of nodes of Hodgkin's disease, injected onto the surface of the chorioallantoic membrane of hen's eggs, produced grape-like clusters of vesicles. Bits of such membrane cultured and stained with brilliant cresyl blue showed the specific cell inclusions found in the original tissue culture of nodes from patients with Hodgkin's disease. No bacterial, fungal, or protozoan infection or contamination was ever observed in the many hundred cultures of nodes from patients with Hodgkin's disease, despite the fact that the tissue culture medium is favorable for the growth of aerobic bacteria.—M. B.

Chromosomal Enlargement in Neoplastic Rabbit Tissues. Bieselle, J. J. [Univ. of Pennsylvania, Philadelphia, Pa.] *Cancer Research*, **5**:179-182. 1945.

An adenocarcinoma of the rabbit uterus was found to have a high frequency of metaphases, chiefly diploid, containing chromosomes about twice as large as those in normal tissues, and this neoplasm also had about 12 plasmosomes in most of its resting nuclei. Since the 4 normal tissues examined seemed to have a maximum plasmosome number of 6 per nucleus, it is probable that the enlarged chromosomes of the adenocarcinoma contained twice as many strands as the chromosomes of normal tissues. Similar enlarged chromosomes were present in lower frequency in another uterine adenocarcinoma and a mammary tumor, but none were found in 2 apparently benign tumors of the rabbit uterus. The rabbit becomes the fifth organism to exhibit chromosomal enlargement by doubling of strands in its malignant cells. Although the evidence is suggestive, the series of tumors examined is too small for definite conclusions to be drawn concerning the value of chromosomal enlargement as an indicator of growth autonomy in tumors.—Author's abstract.

Growth and Pathology of Melanoma S91 in Mice of Strains dba, A, and C. Algire, G. H. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:151-160. 1944.

The gross and microscopic characteristics of melanoma S91 when transplanted are reported. Although the tumor originated in a pigmented JAX-dba mouse, it will now grow progressively in a high percentage of mice of the albino A and C strains as well as in the pigmented dba strain. Metastases, which are themselves transplantable, occur frequently in the lungs, liver, kidneys, adrenals, lymph nodes, and orbits. By selection, an almost pigment-free strain of the tumor also has been developed.—R. A. H.

The Action of Xanthopterin on Tumor Growth. Lewisohn, R., Laszlo, D., Leuchtenberger, R., and Leuchtenberger, C. [Mt. Sinai Hosp., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **56**:144-145. 1944.

A "folic acid concentrate" and the crystalline *L. casei* factor have previously been shown to inhibit tumor growth. Since a possible relationship of folic acid to xanthopterin (the pigment of yellow butterfly wings) has been suggested, the action of synthetic xanthopterin was tested on tumor growth.

Female Rockland mice, 8 days after receiving implants of sarcoma 180, were given 0.031 to 2.00γ of xanthopterin in 4 intravenous injections over a period of 48 hours. Tumor growth was inhibited, the degree of inhibition depending on the dose. The mean terminal tumor weight (866 mgm.) of the controls was more than twice that (389 mgm.) of the group receiving the highest dose.

Leukopterin (the pigment of the white butterfly), tested by the same method, caused no inhibition of tumor growth even in a dose 100 times as great as that of xanthopterin. When the 2 pigments were given in combination, leukopterin neutralized the action of xanthopterin.—M. B.

The Effect of Penicillin and Choline upon the Appearance, Growth, and Disappearance of the Emge Sarcoma in Rats. BEARD, H. H. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Exper. Med. & Surg.*, **2**:286-289. 1944.

The daily subcutaneous injection of 333 Oxford units of a yellow sodium penicillin preparation (Abbott) in 2 cc. of solution, beginning on the day of intra-abdominal implantation of Emge sarcoma, apparently reduced the number of takes and the growth of the tumors and significantly increased the number of tumors that disappeared. Similar effects were exerted by ingestion of 40 mgm. of choline chloride in the drinking water. In the penicillin-treated animals in 1 experiment, 72% of 32 tumors disappeared after starting to develop, and in the choline-treated animals of the same study, 68% of 40 tumors disappeared, as compared with 19% of 32 tumors in controls. In 2 other studies, the disappearance rates for penicillin-treated, choline-treated, and control rats were respectively: (experiment 1) 44, 38, and 0% of 18, 8, and 66 tumors; (experiment 2) 35, 35, and 2% of 32, 54, and 50 tumors. When 333 or 666 Oxford units of penicillin was injected into rats already bearing tumors weighing 40, 105, and 70 gm. respectively, the tumors decreased to 10, 40, and 10 gm. in 5 days, 6 weeks, and 6 weeks, but the animals eventually died. Two other rats bearing large tumors died 3 days after penicillin injections were started. In further experiments, pure sodium penicillin will be used.—M. H. P.

Penicillin Treatment of Crown Gall. BROWN, J. S., and BOYLE, A. M. [Arizona Agric. Exper. Station, Tucson, Ariz.] *Science*, **100**:528. 1944.

Galls produced on *Bryophyllum* by hypodermic inoculation of *Agrobacterium* (*Phytomonas*) *tumefaciens* were apparently completely destroyed by crude penicillin. To bring about this complete destruction it was necessary to puncture the gall in many places and to cover it with cotton soaked with the penicillin solution. The authors comment on the fact that penicillin here apparently destroys a gram-negative organism (the crown gall bacterium), while in general gram-negative bacteria are reported to be relatively resistant to the drug.—R. B.

Chemical Treatment of Tumors. X. Reactions of Four Patients with Advanced Malignant Tumors to Injection of a Polysaccharide from *Serratia marcescens* Culture Filtrate. BRUES, A. M., and SHEAR, M. J. [Harvard Med. Sch., and Massachusetts Gen. Hosp., Boston, Mass., and Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:195-208. 1944.

Four patients with widespread, far advanced neoplastic disease of different types (prostatic carcinoma, lymphosarcoma, multiple myeloma, and Ewing's sarcoma) were treated with injections of a polysaccharide derived from culture filtrates of *Serratia marcescens*. All the patients died with tumors within 6 months of the beginning of the treatment although one patient, and possibly a second, showed some improvement during the period immediately following treatment. The generalized reaction of these patients to the polysaccharide is given. Immediately after the first intramuscular injection all the patients showed a marked elevation of temperature accompanied in 3 cases by a drop in blood pressure to, or approaching, "shock levels," 1 patient becoming anuric and comatose. Two of the older patients developed signs of cardiac decompensation such as pulmonary congestion and peripheral pitting edema. Subsequent intramuscular injections of the polysaccharide produced less severe reactions indicating the development of tolerance to the substance. One patient, who previously had received 4 intramuscular injections and had failed to react to the last of these, died with signs of cardiovascular failure 1½ hours after the intravenous administration of a reduced dose of the polysaccharide. In one case elevated nonprotein nitrogen and uric acid levels in the blood, accompanied by an increased urinary excretion of these metabolites, suggested an increase in tissue breakdown following polysaccharide administration. The authors conclude that, "The findings in this preliminary study do not warrant the conclusion that this polysaccharide should be recommended in the treatment of patients with malignant tumors."—R. A. H.

Attempts to Produce Anaphylaxis in Guinea Pigs with a Polysaccharide from *Serratia marcescens* (*Bacillus prodigiosus*). FRANKE, F. E. [St. Louis Univ. Sch. of Med., St. Louis, Mo.] *J. Nat. Cancer Inst.*, **5**:173-177. 1944.

An attempt was made to produce anaphylaxis in guinea pigs with a lyophilized, protein-free preparation of polysaccharide from *Serratia marcescens*. Varying sensitizing doses ranging from 0.2 to 0.000002 times the L. D. 50 for this preparation, given subcutaneously, were followed 21 to 28 days later by an intracardiac shocking dose. Anaphylaxis was determined by clinical observation and by measurement of lung size and specific gravity at autopsy. Of 64 animals tested none gave a typical picture of anaphylactic shock although 20 animals showed some abnormal reaction. The L. D. 50 was 5.0 mgm./kgm. subcutaneously, 1.0 to 2.2 mgm./kgm. upon injection into the heart.—R. A. H.

Effects of Sublethal Doses of a Polysaccharide from *Serratia marcescens* (*Bacillus prodigiosus*) on the Electrocardiogram, Blood Ascorbic Acid, and Nonprotein Nitrogen of the Dog. FRANKE, F. E., and RICHERT, D. [St. Louis Univ. Sch. of Med., St. Louis, Mo.] *J. Nat. Cancer Inst.*, **5**:179-183. 1944.

The effect of injection of a polysaccharide from *Serratia marcescens* was studied in 6 dogs. No significant change, other than sinus tachycardia, was found in the electrocardiogram after the administration of the polysaccharide. The blood ascorbic acid response varied, levels increasing to a rather pronounced degree in 4 dogs but decreasing a somewhat lesser extent in the other 2. Three dogs exhibited a progressive rise in the nonprotein nitrogen

level of the blood, while 2 others showed little change. Urinary nitrogen excretion was not determined. No significant changes in serum calcium were noted in the 2 dogs tested.—R. A. H.

Action of Toxic Doses of the Polysaccharide from *Serratia marcescens (Bacillus prodigiosus)* on the Dog and Guinea Pig. FRANKE, F. E. [St. Louis Univ. Sch. of Med., St. Louis, Mo.] *J. Nat. Cancer Inst.*, **5**:185-193. 1944.

When lethal doses of a polysaccharide obtained from *Serratia marcescens* were injected into anesthetized dogs, a rapid drop in blood pressure to "shock levels" with a sharp rise in pulse rate occurred, and the animals frequently died from circulatory failure within 1 to 5 hours. Those animals that did not die within this period showed signs of recovery. Unanesthetized dogs reacted quite differently, and death did not occur until 18 to 28 hours after injection. In these dogs severe gastrointestinal disturbances were the first symptoms, followed by prostration and later by convulsions and death. An early rise in rectal temperature and a late drop in blood pressure were noted in these animals. Adrenaline was of little value in combating the signs of "shock," but the intravenous administration of saline appeared to be helpful. Postmortem examination revealed congestion and hemorrhage in the gastrointestinal tract of about one-fourth of the animals, and occasionally also in the spleen, lymph nodes, liver, kidneys, and adrenal medulla. A similar picture was noted, before and after death, in guinea pigs given lethal doses of the polysaccharide. No generalized increase in capillary permeability was observed in rats injected with trypan blue after the administration of the polysaccharide.—R. A. H.

Research Activities of the National Cancer Institute. SHIMKIN, M. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:77-88. 1944.

A review is presented of investigations on cancer conducted by the U. S. Public Health Service during 1922 to 1937 (before passage of the National Cancer Institute Act), and from 1938 to the present. Research at the National Cancer Institute has been concerned with: the experimental production of neoplasms; carcinogenic agents (including chemical compounds and ultraviolet radiation), and the effect of genetics, hormones, and diet upon their action; cellular physiology; chemistry of tumors; experimental cancer therapy; and epidemiology. No effective therapeutic agent was discovered in an empiric assay of 977 substances and mixtures of substances in 18,395 mice bearing transplanted or spontaneous neoplasms. Clinical tests are being made, however, of a highly active, purified polysaccharide, isolated from cultures of *Bacillus prodigiosus;* in doses of 0.1 μgm., this polysaccharide produces hemorrhage in mouse sarcoma 37. Colchicine and anaphylaxis also evoke hemorrhages in transplanted sarcoma of mice; compounds related to colchicine are being investigated. Subcutaneous implantation of diethylstilbestrol is being tried in prostatic cancer in man. It is suggested that future research be guided by the accumulating evidence that cancer is a group of diseases rather than a single disease.—M. H. P.

Experimental Cancer Research in the Soviet Union. SHIMKIN, M. B. *Am. Rev. Soviet Med.*, **1**:43-48. 1943.

The scope and some conclusions of numerous cancer research investigations in the Soviet Union are briefly reviewed with bibliography. Among the reported achievements are the demonstration of carcinogens in the liver and other tissues of patients with cancer, the production of malignant tumors in rats upon transplantation of embryonic tissue, the heterotransplantation of a melanosarcoma of man into the rat, the use of tissue culture technic in the study of the metabolism of neoplastic tissue, the heterotransplantation of tumors upon blockading of the reticuloendothelial system, the stimulation and inhibition of the function of the active mesenchyme by specific cytotoxic antimesenchymal serum, the increasing of carcinolytic activity of the serum of cancer patients by anti-human spleen horse serum, and the demonstration of reduced mitogenetic radiation in the blood of patients and animals with cancer.—M. H. P.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

DIAGNOSIS—GENERAL

On the Demonstration of Neoplastic Cells in the Sputum. WANDALL, H. H. [Radium Station, and State Hosp., Copenhagen Univ., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **20**:485-494. 1943.

Neoplastic cells were demonstrated in the sputum in 19 of 22 histologically verified cases of primary pulmonary carcinoma, and in 7 of 8 roentgenologically positive cases of the same disease. Such cells were also found in the sputum of patients with pulmonary metastasis from osteosarcoma, hypernephroma, carcinoma of the ovary, and seminoma, but not in the sputum of a patient with benign bronchial tumor. One false positive diagnosis of neoplasm, in a person with chronic fibrotic pneumonia, is attributed to inexperience with the technic; 19 cases of nonneoplastic conditions were negative.—M. H. P.

RADIATION—DIAGNOSIS AND THERAPY

Selection of Physical Factors for Maximum Output in Roentgen Therapy. SAUNDERS, W. W. [San Francisco Hosp., and Stanford Univ. Med. Sch., San Francisco, Calif.] *Radiology*, **41**:164-169. 1943.

For obtaining maximum depth dose with limited facilities, the anode-skin distance and filtration must be carefully chosen. A chart for the selection of treatment factors is given.—R. E. S.

Limitations of Physics in Radium Therapy. PARKER, H. M. [Tumor Inst. of Swedish Hosp., Seattle, Wash.] *Radiology*, **41**:330-336. 1943.

This paper is a supplement to the Paterson-Parker charts in radium therapy. The problems and errors that may occur with the present system of distributing radium and calculating irradiation are discussed.—R. E. S.

The Histopathology of Radiation Lesions. WARREN, S. [Harvard Cancer Commission, New England Deaconess Hosp., and Harvard Med. Sch., Boston, Mass.] *Physiol. Rev.*, **24**:225-238. 1944.

This review sums up the more recent information on the biologic effects of radiation, with emphasis on: (a) effects on cytoplasm and nucleus of cells; (b) the relative sensitivity of different mammalian cells and tissues, both somatic and germinal; (c) the sequence of clinical and histologic changes in various normal tissues following radiation; and (d) the sequence of these changes in irradiated tumors of 3 general types—radiosensitive, radioresponsive, and radioresistant.—R. B.

The Pathology of Brain Tumors and Its Relationship to Roentgenologic Diagnosis. BOLDREY, E. [Univ. of California Hosp., San Francisco, Calif.] *Radiology*, **41**:107-116. 1943.

A correlation of the pathology of various types of tumor occurring in the brain with their roentgenographic appearance is presented. Brain tumors are divided into the supratentorial and subtentorial. Of the supratentorial group gliomas comprise 40 to 50%. These tumors arise from the brain substance and pneumographic studies show more or less pronounced ventricular shift. Meningiomas comprise approximately 13% of supratentorial tumors and show a fairly characteristic appearance in their various forms. Other less frequent supratentorial types are the pituitary tumors, craniopharyngiomas, cysts of the third ventricle, pineal tumors, angiomas, and cholesteatomas. The subtentorial tumors, of which the medulloblastoma is most common, may produce signs of increased intracranial pressure and changes in the fourth ventricle with dilatation of the lateral ventricles and third ventricle. Clinical data together with complete roentgen studies can provide a basis for accurate localization. —R. E. S.

Reliability of Brain Tumor Localization by Roentgen Methods. JOHNSON, V. C., and HODGES, F. J. [Univ. of Michigan, Ann Arbor, Mich.] *Radiology*, **41**:117-129. 1943.

A study was made of the various diagnostic methods used for 565 patients with brain tumor seen at the University of Michigan Hospital over a 10 year period. Routine skull examination, encephalography, and ventriculography all played a role in diagnosis. Adequate equipment, facility in its use, and experience are prime requisites if the radiologist is to contribute to the diagnosis of brain tumors.—R. E. S.

Application of Some New Technics to Study of Brain Tumors. WITWER, E. R., DERBYSHIRE, A. J., and CORRIGAN, K. E. [Harper Hosp., Detroit, Mich.] *Radiology*, **41**:130-141. 1943.

Encephalography and petrographic studies are described, and case reports given to demonstrate their value. The EEG is an adjunct to, but not a substitute for, other studies in the localization of brain tumors. The petrographic microscope, which operates on a beam of polarized light, is of value in the study of fresh or formalin-fixed tissues without the alterations caused by staining or other chemical methods.—R. E. S.

Preoperative Roentgen Therapy of Breast Carcinoma: Analysis of Histologic Reaction and Roentgen Technic. DANN, D. S., and KORITSCHONER, R. [Menorah Hosp., Kansas City, Mo.] *Radiology*, **41**:213-224. 1943.

A small group of 10 cases of carcinoma of the breast in which a preoperative cycle of radiation therapy was given is reported. In none was there absolute sterilization of the tumor. At the present time it is concluded that radiation may be given in an attempt to make operable an inoperable carcinoma, when operation is refused, or when other physical conditions contraindicate operation. A central commission to study the best way to treat carcinoma of the breast is proposed.—R. E. S.

Radiation Therapy of Carcinoma of the Breast. PETERS, M. U. [Toronto, Canada] *Canad. M. A. J.*, **51**:335-343. 1944.

The author stresses three main conclusions in a survey of about 176 cases. In the most promising group of cases, Stage I, the 5 year clinical cure rate was 75%, as contrasted with 65% in many larger surgical clinics; this suggests some benefit from prophylactic radiation. The statistics cited in this paper show that postoperative x-ray therapy has raised the survival rate by 15 to 20% among Stage II cases. It is suggested that the prognosis in mammary cancer Stage II might be more noticeably improved by the institution of preoperative radiation in preference to, or in some instances combined with, postoperative treatment. Among the hopeless cases of carcinoma of the breast, Stage III, both primary and recurrent, radiation offers the only hope of relief of distressing symptoms.—M. E. H.

Discussion on the Radiotherapy of Malignant Disease of the Ovary. BREWS, A., ET AL. *Proc. Roy. Soc. Med.*, **37**:720-730. 1944.

MR. A. BREWS reported on the incidence of malignant disease of the ovary, and its pathological and clinical types. He made a strong plea for exploratory laparotomy in the majority of cases.

MISS M. C. TOD reported on radiotherapy of malignant disease of the ovary under five headings: 1. *Types of tumor which the radiotherapist is asked to treat.* 2. *Results of treatment.*—Although no final conclusion can be drawn from the 73 cases reported upon, 50% of the fully treated patients are alive more than a year after treatment. 3. *Principles of treatment.*—Since all of the peritoneal cavity is potentially involved, it is a first principle of treatment that the entire region from the floor of the pelvis to the underside of the liver must be treated. It is necessary to hold the balance between volume of tissue treated and dosage. 4. *Technic of treatment.*—Three fields are used. The anterior fields are rectangles 30 × 20 cm. tilted 30° from the vertical, the applicator being brought down on a simple mechanical "trunk bridge." The posterior field is a circle of 30 cm. diameter directed along the midline. The paper gives details of the resulting radiation distribution. The dose, described as "tumor dose" although it is delivered to the whole central zone, is 3,000 r in 3 to 4 weeks. It is necessary to begin with a low dose and increase each day. 5. *Toler-*

ance of the patient.—Investigation has been made of the blood changes following irradiation, a relationship being sought between integral dose and total white count as well as absolute number of lymphocytes and monocytes. It is shown that the integral dose to reduce white cells to one-half increases with the time over which the integral dose is spread.

Miss Tod concluded that in all cases of malignant neoplasm of the ovary in which removal is incomplete or impossible, the patient should have the chance of a course of x-ray therapy.

Dr. F. Ellis contributed a statistical analysis of 105 cases of primary carcinoma of the ovary treated at the Sheffield Radium Centre from 1932 to 1939. The results shown strongly suggest, but do not prove, that radiation therapy is of value. Details of the results must be sought in the original paper.

Mr. A. Green expressed surprise that such a large volume of tissue as the whole abdomen and pelvis was irradiated *en bloc* to a dosage of at least 3,000 r. In his experience, such a large body dosage makes the patient ill, and it often has been found beneficial to treat the patient in stages, pelvis first and abdomen later, or in strips. It might be an advantage to treat the patient from below because of the mobility of the pelvic structures.

Miss Tod (in reply) stated that they had treated a large number of cases in the manner described and had never had any serious trouble due to the large volume of tissue irradiated.—W. V. M.

An Evaluation of Radiation in the Treatment of Carcinoma of the Corpus Uteri. Corscaden, J. A. [Columbia Univ. Coll. of Physicians and Surgeons, New York, N. Y.] *J. A. M. A.*, **126**:1134-1138. 1944.

Hysterectomy, radium, and x-ray are essential in the treatment of operable carcinoma of the uterus. Hysterectomy alone promises a 5 year survival rate of approximately 60%, and radium alone 55%; x-rays alone are incompletely effective (results in the literature are widely discrepant). When all these methods are employed in combination, a survival rate of 80% may be expected. —M. E. H.

A Review of Carcinoma of the Bladder Treated by Supervoltage X-Rays over a Five-Year Period. Colby, F. H., and Schulz, M. D. [Massachusetts Gen. Hosp., and Colis P. Huntington Memorial Hosp., Boston, Mass.] *Radiology*, **41**:371-377. 1943.

An analysis of patients treated for carcinoma of the bladder by supervoltage x-ray indicates that the palliation accomplished justifies the continuation of this method of treatment. One hundred and thirty-nine cases were studied in which, for various reasons, surgery was not indicated. Symptoms were relieved in over one-half the treated cases, and pronounced regression occurred in about one-third. The low-grade tumors responded less well than the highly malignant, rapidly growing types. In some cases the tumor had shrunk sufficiently to permit the use of other types of treatment in conjunction with the external radiation. It was felt that the patients were made more comfortable and lives probably lengthened by the treatment.—R. E. S.

Carcinoma of the Trachea: Report of Two Cases Diagnosed and Treated by Roentgen Rays. Brown, S., Weiss, H. B., Iglauer, S., and Fine, A. [Cincinnati, Ohio] *Radiology*, **41**:394-397. 1943.

Two cases of carcinoma of the trachea are reported. Both tumors were demonstrated by roentgenography in the oblique or lateral position with a barium-filled esophagus.—R. E. S.

Roentgenographic Diagnosis of Neoplasms of the Peri-Ampullary Region and Head of the Pancreas. Brunschwig, A., and Templeton, F. E. [Univ. of Chicago Clin., Chicago, Ill.] *Radiology*, **41**:438-443. 1943.

The increasing success of radical surgery in the treatment of carcinoma of the ampulla of Vater and head of the pancreas makes roentgen diagnosis of these lesions of increasing importance. Large polypoid tumors in the lumen are readily observed if the duodenum can be distended. Irregularity and stiffness of the duodenal walls may be produced by ampullar carcinoma. Extensive disease with infiltration of the duodenal wall may produce marked changes in the mucosa. Frostberg's reversed "3" sign when present may be a valuable aid to diagnosis. Lesions of the head of the pancreas may or may not produce definite roentgenographic changes. Clinical study is of aid in the differential diagnosis.—R. E. S.

Roentgen Examination of Pancreatic Tumors. Borak, J. [New York Univ. Med. Coll., New York, N. Y.] *Radiology*, **41**:170-180. 1943.

The various roentgen methods for the study of pancreatic tumors are discussed. Usually the diagnosis must be made by a combination of various procedures available, *e.g.* fluoroscopy of the chest, roentgenography of the spine and abdomen, cholecystography, visualization of the pancreatic duct, and roentgenography of the gastrointestinal tract after administration of barium preparations or inflation with gas.—R. E. S.

Giant-Cell Tumors: Radiation Therapy and Late Results. Gershon-Cohen, J. [Philadelphia, Pa.] *Radiology*, **41**:261-267. 1943.

A review of 29 cases of giant-cell tumor seen at the University of Pennsylvania Hospital indicates that irradiation produces good late results. Moderately large doses were given, and the patients appeared to do better when weight-bearing was avoided during healing. The phenomenon of apparent increase in the tumor with osteoclasis, swelling, and pain was sometimes observed after radiation given before healing took place.—R. E. S.

An Apparently Solitary Myeloma of Bone with Subsequent Generalization. Toth, B. J., and Wintermantel, J. A. [St. Francis Hosp., Olean, N. Y.] *Radiology*, **41**: 472-477. 1943.

A case of myeloma of the pubic bone with subsequent generalization is presented with a differential diagnosis. Comparatively small doses of x-ray treatment led to local and general improvement but caused severe chills and fever.—R. E. S.

Protection in Radiology. An Exhibit. Quimby, E. H., and Pool, J. [Memorial Hosp., New York, N. Y.] *Radiology*, **41**:272-281. 1943.

The authors present tables and charts covering the various phases of protection in x-ray and radium therapy. —R. E. S.